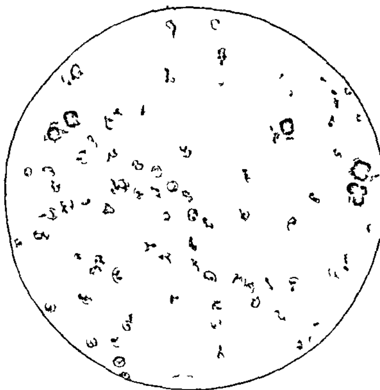


Illustrating Professor Ross's method of making thick films for the detection of parasites when scanty in the blood

Method — *A drop of blood is spread out on the slide over an area that would be covered by a sixpence. Dry thoroughly. Place in water. Then without fixing stain in the ordinary way with Romanowsky*

Frontispiece

THE PRACTICAL STUDY OF MALARIA

AND OTHER BLOOD PARASITES

J W W STEPHENS MD CANTAB D P H

W LT M CT T A M
T

AND

S R CHRISTOPHERS MB VICT I M S

M M T C T MM SS M A
898 9

PUBLISHED FOR
THE UNIVERSITY PRESS OF LIVERPOOL

LONGMANS GREEN & CO
39 PATERNOSTER ROW, LONDON
NEW YORK AND BOMBAY
1903

At the University Press of Liverpool
No 46 Nov 1903 500

CONTENTS

CHAPTER I

Preparation of wet and dry blood films Fixing staining Romanowsky stain

CHAPTER II

Normal blood Varieties of leucocytes Platelets Abnormal constituents

CHAPTER III

Forms of the malaria parasite commonly seen in blood films Rings large forms crescent and spherical bodies Pigment (melanin) Flagellation Distinction of species simple tertian malignant tertian and quartan Gametes

CHAPTER IV

Subsidiary signs of malaria Pigmented leucocytes An increase in the percentage of large mononuclears Normal leucocytic values

CHAPTER V

Examination of tissues Smears Simple apparatus for embedding in paraffin

CHAPTER VI

Life history of malaria parasite Explanation of terms

CHAPTER VII

Mosquitoes *Diptera* Life history of mosquitoes *Culicina* and *Anophelina* Capture of mosquitoes

CHAPTER VIII

Examination of eggs of *Stegomyia* *Culex* *Taeniorhynchus* *Anopheles* etc

CHAPTER IX

Larva and nymph Respiratory syphons of larva and nymph generic differences

CHAPTER X

Feeding mosquitoes on man on birds Breeding out mosquitoes

CHAPTER XI

Dissection of mid gut viscera etc Detection of zygotes Salivary glands and sporozoits To cut sections of mosquitoes

CHAPTER XII

Internal anatomy Histology

CHAPTER XIII

To collect and preserve mosquitoes

CHAPTER XIV

External anatomy Proboscis and palpi Escape of sporozoit and filarial embryo Thorax Wing venation Legs

CHAPTER XV

Classification and identification of the *Culicidae* Scale structure

CHAPTER XVI

Classification and identification of *Anophelina*

CHAPTER XVII

Anopheles Habits distribution hibernation dispersal Domestic and wild species Food Fecundation

CHAPTER XVIII

Anopheles Examination of eggs

CHAPTER XIX

Anopheles The larva Clypeal and palmate hairs The nymph

CHAPTER XX

Anopheles Breeding places Selective action of species

CHAPTER XXI

Anopheles Identification of larvae Larvae of Indian *Anopheles*

CHAPTER XXII

Anopheles Relation of specie to malarial endemicity

CHAPTER XXIII

Endemic malaria Endemic index Mode of determination Endemic areas of a country

CHAPTER XXIV

Clinical study of malaria The leucocytes The urine *Determination of cycle of development of a parasite* *The* absorption and elimination of quinine Post mortem change

CHAPTER XXV

Blackwater fever Examination of the blood and urine Post mortem changes

CHAPTER XXVI

The haemamoebidae Proteosoma halteridium parasites of bats monkey cattle reptile The haemogregarine of reptile fish etc The piroplasmata Texas fever African Coast fever Spotted fever etc Ticks anatomy and classification The yellow fever protozoon *Stegomyia fasciata* Spirillar fever and tick

CHAPTER XXVII

The trypanosomata *T rotatorium* *T carassii* *T eberthi* *T Cobitis* *T lewisii* *T Brucei* (Nagana) tsetse flie *T evansi* (Surra) *T equinum* (Mal de Caderas) *T equiperdum* (Dourine) *T gambiense* (human) *T ugandense* (sleeping sickness) *T theileri* *T transvaaliense* The trypanoplasmata

CHAPTER XXVIII

Filaria human mammalian avian

APPENDICES

The biting flies and fleas Stains Weights and measures Apparatus

PLATE I*

Human Trypanosome

Fig 1 —Human trypanosome in Gambian native × 2 000
Fig 2 —Human trypanosome in tame rat long form × 2 000
Fig 3 —Human trypanosome in tame rat showing longitudinal division × 2 000
Fig 4 —Human trypanosome in tame rat from a specimen taken one week before death stumpy form showing chromatin granules × 2 000
Fig 5 —*Human trypanosome* in tame rat from a specimen taken one week before death round form showing granules × 2 000

Gambian Horse Trypanosome

Fig 6 —Horse *trypanosome* The small tadpole shaped parasite in the early stage of the disease × 2 000
Fig 7 —Horse trypanosome stumpy form in tame rat × 2 000
Fig 8 —Horse trypanosome showing longitudinal division of tadpole shaped parasite × 2 000
Fig 9 —Horse trypanosome long form showing longitudinal division × 000
Fig 10 —Horse trypanosome long form

PLATE II

Senegambian Trypanosomes

Birds

Fig 1 —Trypanosoma *Johnstoni* n sp From the blood of *Estrelda estreld* × 000
Fig 2 —Trypanosoma sp incert × 000

Frogs

Fig 3 —Trypanosoma rotatorium × 2 000
Fig 4 —Trypanosoma mega n sp × 2 000
Fig 5 —Trypanosoma n sp × 000 Showing chromatic granules extending in a chain from the centrosome to the nucleu

---

From th t th m rep t of Drs D tt n I T d l n Tryp m

their own microscopes, follow all the most recent work on Malaria and eventually be in a position themselves to add new facts to our knowledge of this important disease

For instance, with very little apparatus it is possible to undertake many most important researches, *e g*, to work out the rationale of infection in any station or cantonment, the form of the parasite present the percentage of adults and children infected the species of *Anopheles*, where each species is found and where it breeds the percentage of each species carrying sporozoits and zygotes

In fact nearly the whole technique of Malaria can be conducted with a microscope, a few slides and coverglasses, a needle a stain some tubes, pins and cardboard (*Vide* Appendix)

While our original intention was to write a practical guide to Malarial Study solely, yet the opportunities for research on other blood parasites are so numerous in the tropics that we have thought it to be of practical value to add short supplementary chapters on other Hæmatozoa and on the Trypanosomidæ, etc

their own microscopes, follow work on Malaria and eventu themselves to add new facts t this important disease

For instance, with very li possible to undertake many searches, *e g*, to work out the r in any station or cantonment, site present the percentage of infected the species of *Ano* species is found and where centage of each species carr zygotes

In fact nearly the whole can be conducted with a mic and coverglasses, a needle, pins, and cardboard (*Vide*

While our original inten practical guide to Malarial opportunities for research on are so numerous in the tro thought it to be of practica supplementary chapters on ot on the Trypanosomidae etc

The Practical Study of Malaria

Chapter I

TO PREPARE BLOOD FILMS

FOR ordinary work we have no hesitation in saying that it is better to use [illegible] dry films The advantages of using these are many —

1 They are much less trouble to make especially under the adverse conditions one has to contend with in the tropics

2 They need not be looked at at once but can be put aside until one has the leisure to examine them

3 Large numbers fifty or one hundred may be taken at once as will be found constantly necessary and examined weeks or months later a thing quite impossible with wet films

4 They give information as to the leucocytes if this is needed and may be examined over and over again when some new point of view demands a re examination

5 By the use of exceedingly simple methods the routine of blood examinations may be reduced to the greatest simplicity

For studying movement delicacies of structure for watching the process of exflagellation

B

phagocytosis fertilization, and other phenomena of the living parasite, it is necessary, however, to be able to make good wet films and the points of importance in the preparation of these will be described

The Preparation of Dry Films

The simplest and by far the best way of making films is by the use of no other apparatus than—

1 A straight surgical needle about two inches in length
2 Clean glass slides

To Clean Slides

Slides should be dipped in water and rubbed dry and clean with a soft cloth *e g*, a clean handkerchief To ensure the best results it is well to heat the slides in the flame of a spirit lamp, or smokeless paraffin lamp and allow them to cool on a sheet of clean paper For ordinary purposes this is quite unnecessary But if a perfectly clean slide is required then heat it red hot over a flame In this way grease is completely removed

Before proceeding to take specimens of blood the prepared slides may be placed in a small pocket slide box or wrapped in a sheet of clean dry white paper A packet of half a dozen prepared slides wrapped in a sheet of note paper, afterwards transfixed with the needle is a most convenient form of carrying the necessaries for taking specimens of blood The needle should be an ordinary triangular pointed, straight surgical needle (It is best to nip off the eye with pincers)

To Clean the Patient's Finger

If the finger of one's subject is obviously dirty and especially if damp with sweat, the finger should be roughly wiped with a cloth. If considered necessary precautions may be taken to avoid all skin contaminations by the routine of water, alcohol and ether, but in ordinary examinations for malarial parasites this is quite unnecessary.

To Prick the Finger

The last phalanx of the finger (the third finger of the right will be found most convenient and the skin usually soft) is taken between the finger and thumb of the left hand of the operator and *gently* pressed to force the blood towards the pulp. A slight prick with the triangular pointed needle will in most cases cause a fair sized drop of blood to exude.

To Make the Film

When the drop of blood reaches the size of the head of a small pin, a slide is taken in the right hand and lowered on to the drop (taking care not to dab it on the skin). If the drop is too large wipe it away and squeeze a small fresh one. The drop should be transferred to the slide about one third inch from the far end. The slide is then changed to the left hand, the finger and thumb grasping the end nearest to the drop. The right hand again takes the needle and holding it by the pointed end lays the cylindrical shaft transversely to the slide and across the drop of

blood After waiting about a second that is until the drop spreads to the extent of about one third inch between the slide and the needle, the needle is evenly and not too quickly carried to the right and so along the whole length of the slide The right amount of pressure is very

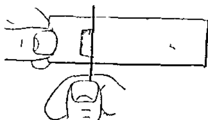


Fig 1 *Authors method of making blood films*

quickly learnt and the making of a useful and good film is far easier in this way than in any other known to us (Fig 1) Immediately the film is made it should be waved to and fro until it is seen to be quite dry The quicker the film dries the more perfectly preserved will be the red cells of the blood

THE CHARACTERS OF A GOOD FILM

1 As a *film is needed to detect* even the most minute forms of the parasite within the red cell it is necessary that the red cells be well spread out and not distorted or lying over one another To ensure this the film must be uniform and as thin as possible

2 Films should be made so that, if desired, the leucocytes may be differentially counted A

little practice will enable one to make films with the upper and lower edges more or less parallel with the edges of the slide and terminating in a pointed manner about half an inch from the right hand end of the slide Practically the whole of the drop of blood is then upon the slide and the edges to which the leucocytes tend to find their way are in a suitable position for examination (see differential counting of leucocytes p 41)

3 In the case of very anaemic bloods *e g*, those of malarial cachexia difficulty will arise from the film being too thin The needle in this case must be carried very loosely and rapidly along the slide and a thicker film thus made When blood with difficulty adheres to the slide good evidence of extreme anaemia is obtained

The Preparation of Wet Films

A wet film is not so easy to make as a dry film and requires cleanliness and rapidity of manipulation Wet films are therefore difficult to make in dusty countries where a single particle of grit will mar the process

Before proceeding to make films several glass slides and coverglasses should be carefully cleaned and polished with a dry pocket handkerchief and wrapped in clean smooth paper to ensure the absence of dust or grit In making a wet film the result may be marred by—

1 Too small or too large a drop of blood

2 Too slow manipulation allowing the drop of blood to partially clot

3 An uneven coverglass or a coverglass with a minute bubble or speck in its substance

4 Dust of any kind

To Make a Wet Film

The same procedure is gone through as in the case of a dry film It is well to polish the slide and coverglass immediately before use with a clean handkerchief When the exuding drop of blood reaches the size of a *small* pin's head, a coverglass *is picked up rapidly with forceps, or the edges* are grasped between finger and thumb, and allowed to touch the drop without dabbing the skin and again rapidly placed drop downwards upon a slide A gentle tap or two with a needle or forceps may aid in the film formation but the pressure must not be great or the corpuscles will be found haled and invisible

The requirements of a suitable wet film are — There should be a central transparent area shewing no sign of a granular appearance, and even looking quite free from blood If this appearance is present the film is probably a good one If the centre of the film appears reddish or granular, it is useless to examine it (for young parasites) as the corpuscles will be massed together and the parasite not seen Under the microscope a good film should shew clear even circular discs lying side by side and not overlapping each other It may be necessary to make several before a sufficiently good one is obtained

To Label Films

Films should always be labelled as soon as possible otherwise uncertainty and annoyance are sure to arise The use of paper labels is not at all *necessary in routine work*

1 The most convenient method is that of writing on the end or back of the slide with ordinary ink This should be quite dry before placing in alcohol There is then no fear of its coming off

2 An excellent and extremely simple method of labelling has been described by Dr POWELL (Bombay) viz — After making a dry film as described above the name date and other necessary information are scratched on the film with the head or point of the needle The films used being extensive the writing in no way injures them The first half inch or so of the film is frequently rather thick and much information as to name date temperature etc may safely be written on it

To Store Films

Slide boxes may be used holding the slide vertically These should be well cleaned out if made of wood otherwise fine sawdust accumulates on the slide A size which will go in the pocket and holds about twenty five slides will be found a great convenience for daily work Larger boxes to hold one hundred or so are best for use at home half a dozen of these may be enclosed in a stronger outside case In a square foot of space something like 1,500 slides can be stored in this way

If no box is at hand films may be wrapped in clean white paper a fold of paper being placed between each slide For transmitting half a dozen films or so this is quite the most convenient way the whole of course being packed in a box or tin with wool

Both fixed and unfixed films rapidly develop moulds in damp and hot climates Moulds appear as branching threads under the microscope A certain amount of mould does not much interfere with the utility of films if they are intended only for detecting the presence of parasites, counting leucocytes, etc

Unstained films kept for six months or more stain as a rule badly, and are not of much use Stained films (as we shall see, without putting on Canada balsam and a coverglass) will shew excellent results after many years Films that have been unfixed at the time of making and that may have subsequently become damp, will have their red cells destroyed, but the parasites will still stain

The method of wrapping ones slides up in paper (after duly labelling) is in practice the most convenient one especially if travelling, when not unfrequently boxes of slides suffer much damage

To Fix Films

Until a film has been 'fixed it is soluble in water and will be immediately washed off if placed in water or any *watery* solution of a stain *When fixed it is insoluble, and may be treated in* almost any way without destruction Except when using LEISHMAN'S combined fixing and staining solution the film must always be fixed

On returning home or to the laboratory place the films in a glass stoppered cylindrical jar about four inches high and one and a half inch diameter containing absolute alcohol When a number of slides are taken they are placed in

known order and a blank one added *at the last* to avoid the possible mistake of reversing the series. A few minutes is all that is necessary if one is in a hurry. Otherwise they may be left in from one half to twenty four hours as may be convenient. They are then taken out, allowed to dry, and placed in series in a slide box and labelled as desired.

There is the possibility that absolute alcohol may not at times be obtainable. Other fixing methods are —

1. Methylated spirits. (This is in fact quite adequate for the fixing of blood films in the absence of absolute alcohol.)
2. By the use of LEISHMAN'S stain. (The stain itself contains the fixative.)
3. By heat. Heating to $115^\circ - 120$ for half an hour to an hour on a copper plate gives very beautiful leucocyte preparations with EHRLICH'S stain. This method requires care and a copper plate for heating the slides. If greatly pushed one may often satisfactorily fix by passing the slides several times through the flame of a spirit lamp.
4. For other methods *vide* Appendix.

TO STAIN FILMS

A large choice of methods is usually given for the demonstration of the malarial parasite in blood. There is one stain however so much more strikingly effective and generally satisfactory than other stains that for routine use no alternative method need be considered. This stain is ROMANOWSKY'S chromatin stain; the modifications of LEISHMAN will also be found simple and certain.

In some circumstances the one will be found more convenient, in others, the other Both methods are therefore given The making up of the stain although apparently rather complicated is not in reality so and the staining of blood films is one of the simplest most rapid, and certain of methods It is useless to attempt to prepare the stain *unless suitable methylene blue* and eosin are obtained These stains are very inexpensive, and the only care necessary is to order the exact stain from a good firm (C BAKER 244 High Holborn, London)

By the use of Method 2 the staining of blood films is so simplified that a bottle of stain and a supply of water is all that is necessary for the process Process No 1 however is in some ways very *convenient* and *rather more certain* and was largely used by us

Method 1 The following materials are necessary for the making of the stain, viz —

Medicinal methylene blue

Eosin extra (B A or A G) or simply pure eosin for blood staining

Sodium *carbonate*, pure

Two stock solutions are made —

Solution A	Methylene blue	1 0 part
	Sodium carbonate	0 5 parts
	Water	100 parts

The solution is then placed in a hot incubator or by the kitchen fire or in the tropical sun for two or three days By *this time a deep purple* colour will be noticed at the edges of the liquid The colour depends upon the formation of a new red body which combined with eosin forms the active staining principle of ROMANOWSKY Until

the purple colour is developed the solution is quite useless

Solution B Eosin 1 part
Water 1 000 parts

For staining these stock solutions are diluted one in twenty respectively with water *i e* five parts of the stock are made up to one hundred parts with water

To Stain—Equal portions (about four c c) of each solution are poured into a porcelain or any other convenient dish Mix by shaking and put the slides in immediately The amount of stain required, once measured may be marked on a glass tube with a piece of gummed paper and is thus always ready On rocking the solution in the dish a red stain will be seen at the sides this indicates that the staining is proceeding well

Leave the slides in the stain any time from ten minutes to half an hour or longer Wash off the excess of stain with water and allow them to drain or dry them with blotting paper but do not dry them by heating over a flame The red corpuscles may have a bluish tinge This can be got rid of if desired by washing in water or very rapidly in equal quantities of spirit and water

Placed under the microscope while still wet the blood platelets should appear as ruby red granular masses if they are bluish the film should be replaced in the staining solution

Slides may be decolourized to any required extent by soaking in water in fact if left long enough the stain is entirely washed out Such a specimen can however easily be stained a second time

The exact position and relations of pigment are best seen in specimens lightly stained as deep ROMANOWSKY staining may completely obscure pigment

Method 2 LEISHMAN'S stain —

LEISHMAN'S stain consists of the product of interaction of the eosin and the methylene blue of the first method

Make the following solution, viz —

LEISHMAN'S stain	0 15 grammes
Methyl alcohol	100 0 c c.

Place sufficient solution on the slide to cover the film, and allow it to stand for about half a minute Add about twice as much water Mix by moving the slide to and fro, or stir gently with a glass rod Allow it to stain five minutes or longer

The stain is also sold in the form of 'soloids' (by BURROUGHS WELLCOME & Co), each 'soloid = 0 015 grammes If it is impossible to procure methyl alcohol (pure), dissolve the 'soloid in methylated spirit (ten c c) and proceed as above The results got with methylated spirit are perfectly satisfactory for diagnostic purposes This is a most convenient stain, as a tube of soloids and some methylated spirit is all that is required There is no preliminary fixing

After some minutes the slide will be stained The same red scum and precipitate are seen as in Method 1 and are of the same significance The slide should, when stained be washed in water and allowed to remain in this for a minute or so This intensifies the ROMANOWSKY staining and removes the remains of the deposit The red cells also by this process are changed from greenish to faint pink

To obtain the most brilliant results with these stains is perfectly easy and no one who has used them will, except for special reasons use any others at present in use

The advantages of the ROMANOWSKY stain (either method) as stated by LEISHMAN are —

1 The great beauty and brilliancy of the staining

2 The greater certainty of the detection of young forms of the parasites

3 The ease of application and certainty of result

4 The staining of the red cell in simple tertian (SCHÜFFNER s dots)

In malignant tertian also a peculiar staining of the red cell is sometimes seen especially in overstained specimens not washed too much It consists of coarse blotches or clefts and the cell also as a whole stains a deeper tint than the surrounding cells

Chapter II

NORMAL BLOOD*

It is difficult without considerable experience to know exactly the interpretation to put upon many appearances seen in blood films under the microscope The less carefully the film is prepared, the more numerous are artifacts and various contaminations all broadly included under the designation dirt and until one is used to recognize these mistakes from this cause are extremely likely to happen There is no way to get over this difficulty except by experience After a time however artifects and dirt can never be mistaken for a parasite

We may point out some of the artificial appearances that may be encountered —

1 If in any portion of a film the red cells shew as double outlined circles or have a central spot or indeed give any other appearance than

---

I m g t d ap me —
C e rr r
2 The I d aph gm p tly cl d
3 Th co d r m y be r ked d w d p sed w th
F t d p im —
1 Th c l c k d p
2 Th fl t m r
3 Th I d ph cm lm t w d pen.
Alw y k ep ye pe n th t be f th m cr sc p f l s s t th other nd to prev nt d t gett g i l y w p th l off th o l mm s l s w th l ttl yl l b f e p tt g w y A l mm l gen lly k ept q w ll n th tr p s e p lly if in u

that of perfectly uniform disks this portion of the film should be passed over If the whole of the film is of this character it should be discarded

2 It is well to have had the parasite demonstrated to one both fresh and stained After this little or no difficulty will be experienced in recognizing the parasite when it is really present Should there be any doubt the object seen is probably *not a parasite* A parasite stained properly by the ROMANOWSKY stain has always a *blue body* with a *bright* red dot or dots and a more or less clear unstained whitish (vacuolic) area Its *definite* outline whether circular or elongated should be quite clearly made out—then no possibility of mistake should arise

3 Artificial bodies in the red cells are generally to be detected by their occurrence in nearly every corpuscle in one portion of the field and not in another When a really well spread portion of the film is reached they are no longer seen A common artifact of this nature is a small granular mass stained reddish apparently in the red cell It is caused by the staining of vacuoles in the cell The *body* has a granular appearance but the *blue body* red spot unstained area and clear cut outline of the parasite are quite wanting

4 In fresh specimens crenations or vacuoles may simulate young ring parasites It is however impossible to get a clearly defined edge to these by focussing Crenations appear as black dots in one focus and as bright dots in another Vacuoles have not the peculiar look of young parasites and cannot be clearly focussed

5 Masses of bright yellowish brown pigment derived from the skin are common in films made

without cleansing the finger They have no relation, however, to any surrounding parasite Micrococci yeast etc, are commonly found in blood films, especially in the tropics Further, we have found from much experience that the commonest bodies to mistake for parasites are platelets and strange to say leucocytes Platelets (stained)

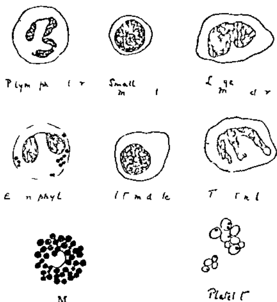


Fig 2 Normal constituents of Blood —
M = Mast Leucocyte

may show a great variety of shape They may be sausage shaped, oval, or elongated, or they may vary in size from about one fifth of a red cell to masses three or four times, or even more as great

as the red cell Frequently, too platelets are surrounded by what looks like a definite clear outline but a closer examination will show that the resemblance to a parasite is only superficial The mass is *granular* throughout a parasite is not The staining is uniformly reddish or bluish blue and red it is not divided as in the parasite between a definite blue area and a definite red dot or dots

Leucocytes we have said are also not uncommonly taken for the larger forms of parasites (*e g*, gametes), but only a beginner could possibly make such a mistake as the leucocytes have a large densely staining mass of red (the nucleus) forming a considerable proportion of the whole cell mass whereas in the gametes there is only a patch or so of red amidst the blue

Dust on the eye piece is *at once detected* by rotating the eye piece when the body shifts its position

THE NORMAL CONSTITUENTS OF THE BLOOD

Normal blood should be carefully studied in fresh and stained specimens

1 *The Red Cells* With Romanowsky's stain these are only faintly stained reddish (Method 2) greenish or bluish in colour (Method 1) Their apparent size *i e* the area they occupy when flattened out depends upon the thickness of the film in well made thin films they are large and stain with beautiful uniformity In fresh specimens (wet films) they ought to appear as perfect uniformly straw coloured discs if crenated it is impossible for the beginner to detect parasites

2. *Leucocytes.*—The following types should be clearly made out in stained films:—

The Polymorphonuclear Leucocytes (Fig. 2).—These are very characteristic, and a reference to the diagram will make their recognition easy. It will be noted that they have a very irregular nucleus and fine granulations (stained red by Romanowsky).

The Small Mononuclear Leucocytes (Fig. 2).—These, the lymphocytes, are readily seen and can scarcely be mistaken for other forms. Their appearance varies somewhat with the thickness of the film. The thinner the film the larger they appear and the greater the area of protoplasm surrounding the nucleus. In typical forms the nucleus is dark staining and nearly spherical.

In wet films a proportion of these cells shew a dark refractile spot (Manson's spot) which might be mistaken for a pigment granule, but, as we shall see later, they cannot possibly be confused with a typical pigmented leucocyte.

The Large Mononuclear Leucocytes (Fig. 2).—It is well to get thoroughly familiar with the appearance of this type of leucocyte, as the percentage of these is of great diagnostic importance in malaria.

Typical large mononuclear leucocytes are readily and clearly distinguishable. They are the leucocytes which may contain malarial pigment, and the recognition of a pigmented leucocyte gives a clear idea of their characters.

(i) They are in thin films of considerable size—half as much again to twice the size of the small.

(ii) The nucleus is large, oval, eccentric, not nearly so dense as in the case of the small—as is

shown by its less intense staining They also present indentations giving a partly bi lobed appearance

(iii) The area of protoplasm surrounding the nucleus is considerable It is clear and contains at most a few scattered granules (ROMANOWSKY stain) The only difficulty will be found to arise in the case of a comparatively small number of 'intermediate' and transitional leucocytes

Intermediate Leucocytes (Fig 2) — These are forms intermediate between the large and small mononuclear forms They are usually classed along with the large forms the characteristics of which they generally more nearly approach

Transitional Leucocyte (Fig) — These are very characteristic and when seen will be at once recognized In shape the nucleus approaches that of the polymorphonuclear forms being trident shaped or S shaped In consistence however it is obviously related to the nuclei of the large mononuclear cells As a rule these cells are small in number and from their close resemblance to the large mononuclears may be included with these

Eosinophil Leucocytes (Fig) The large granules with which these are packed suffice to distinguish them The granules are stained pink or blue (peripherally) by ROMANOWSKY The nucleus in the eosinophil cells is frequently characteristic consisting of two spherical portions united by a thin strand of nuclear material it is really of the polymorphonuclear type

Mast Leucocytes (Fig - M) These in a film stained by ROMANOWSKY are cells crowded with granules stained deep blue or nearly black

These cells occur as isolated specimens in normal blood. They form about 0.5 per cent of all white cells.

3. *Platelets* (Fig. 2).—Bodies of various sizes up to one third diameter of the red cell, nearly always lying in clumps of from six to fifty, and stained bright crimson. They often show a considerable amount of differential staining, but differ entirely in appearance from parasites, more especially in having no *blue* stained mass. An isolated platelet lying upon a red cell may simulate a parasite. For the difference between it and a parasite *vide* above. Platelets often occur in large numbers in cases of malaria and, perhaps, especially in black water fever.

4. *Blood Dust or Granules.*—Small granules, smaller than micrococci. In fresh films they exhibit active motion (? Brownian).



Fig. 2A

Among abnormal constituents of blood we may mention:—

1. *Nucleated Red Cells.*—In conditions of loss or destruction of blood cells, *e.g.* malaria, it is common to see nucleated forms of the red cell in the blood. They are characterized by a small globular nucleus with sometimes one or more little buds staining almost black with Romanowsky. If the

film be counterstained with eosin the fact that the surrounding pale area is red cell will become evident

Two forms may be seen —

(a) Normoblasts *i e* nucleated red cells the size of a red cell (Fig 2A)

(b) Megaloblasts *i e* nucleated red cells much larger than a red cell (Fig 2A)



Myelocyte

Fig 2B

Normoblasts are the form usually seen Megaloblasts in excess are found in pernicious anaemia

2 *Deformed and Small Red Cells may be seen* —These are known as poikilocytes and microcytes They are common in severe anaemias especially pernicious anaemia It is quite exceptional to find deformed cells in blackwater fever The red cells are generally quite normal in shape though anaemic in varying degree

3 *Abnormal Leucocytes* —Under certain conditions *e g* malaria but especially myelogenous leukaemia abnormal leucocyte forms are seen which normally are only found in the marrow *i e* myelocytes These belong to the large mononuclear class and may be of two kinds either with large eosinophil granules as in the eosinophil cell or fine neutrophil granules as in the polymorphonuclear leucocytes (Fig 2B) If large

mononuclear forms are seen crowded with granules films should be stained with Ehrlich's triacid stain, in order to accurately determine the forms of leucocyte present

4 Frequently in malaria films (stained) large open meshworks of nuclear matter are seen with little or no surrounding protoplasm These are degenerated or dropsical leucocytes, and often occur in great numbers

5 *Red Cells with Long Wavy Processes* —These are seen especially in anaemic bloods after the fresh film has been under examination for some time They occasionally break off and float about Shorter and more granular processes emitted by the red cell are even commoner

6 Further we must point out an extra ordinary appearance of the red cells in stained films so far as we are aware not hitherto described In anaemic (malarial) bloods we find red cells ten, thirty, or forty times the diameter of a normal cell and these huge swollen structures shew at one side a crescentic area which is granular, and is the only remaining part of the red cell that can be recognized the remainder is practically un stained These gigantic structures may or may not be occupied by parasites

Chapter III

THE DETECTION OF THE MALARIA PARASITE

EXAMINING THE FILM

After staining and drying the film is ready for examination. *No Canada balsam or cover-glass need be applied.* A drop of cedar wood oil is placed upon the film and the oil immersion lowered into it.

After the examination is completed, if it be desired to keep the film, the cedar oil is dissolved off by dropping a little xylol over the film and allowing this to drain off and then to dry. After drying the film can be put away and kept indefinitely. If not needed the slide is placed on one side with others and eventually cleaned.

TO CLEAN DIRTY SLIDES

1. Rub with turpentine (benzine or xylol) to remove any adherent oil.
2. Wash with soap and water.
3. Rinse in water.
4. Dry and rub well with a clean cloth.

THE DETECTION OF THE MALARIA PARASITE

We propose to describe first the actual appearances which are likely to meet the eye, and later to give a systematic description and mode of distinguishing the various *forms of parasite*. A stained specimen (ROMANOWSKY) should always be used for the purpose of making a diagnosis.

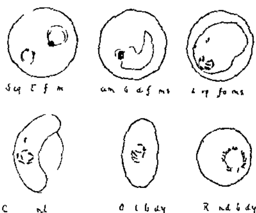


Fig. 3. Forms of the malaria parasite commonly met with in the blood.—The dark dots in the first line represent chromatin, the fine dots pigment.

We may first note that it is not necessary, as is often thought, to examine the blood at any particular time, but it is very necessary that the patient should not have taken quinine previously. Even five grains of quinine may so diminish the number of parasites as to make detection a laborious task, and a negative result under these conditions is not conclusive.

In examining the slide it is a very convenient method to begin at the edge of the film and to work systematically towards the tail end.

The following forms of parasites may be seen:—

(i) Small forms looking more or less like rings or stained streaks lying across or apparently stuck to the side of the red cell.

N.B.—Parasites free in the plasma are practically never seen.

(ii) Larger stained bodies of various shapes and sizes more or less filling the cell.

(iii) Crescents or large round or oval bodies with a cluster of coarse pigment placed more or less centrally.

1. *Ring Forms* (Fig. 3).—These may be quite small, one sixth of a red cell in diameter, or much larger, one third in diameter.

Rings are parasites of very distinct outline and structure. The part of the parasite that will first be noticed in a Romanowsky specimen will be the red nucleus (chromatin), a clearly stained bright red dot (or dots). This is generally situated on the margin of the blue ring, which is equally distinct in outline though often only a faint blue. The blue ring encloses an unstained vacuolic area. These rings stand out so sharply that they appear to project from the corpuscles. The red dot generally forms the signet of the ring (signet forms), but also may occur in the centre of the vacuole. The red nucleus or dot is often also rod shaped or angular. The rings may shew a very faint blue outline or a thicker portion on the side opposite to the nucleus.

Though generally called 'rings' these parasites are really discs, or saucer shaped bodies, adhering to the sides of the red cells

Besides these young rings, we have irregular forms of considerable variety, *e g*, a mere faint bluish line stretching across the corpuscle yet always shewing somewhere a red nucleus, or a mere streak along the margin of the red cell, with, however a red nucleus in the blue protoplasm (accolé forms)

Finally, no small structure should be diagnosed as a parasite unless it is clearly made out that it has three distinct parts

(i) A red nucleus
(ii) Blue protoplasm or body
(iii) A vacuolic area within the ring (in the irregular forms this cannot be distinguished)

No confusion can then possibly arise with a platelet or stained vacuole or dirt

A nucleated red cell has not these characters Nor again, has a red *cell shewing polychrome or* basophil staining *i e* a purplish or bluish mottling all over In fact no other body has the definite quite easily distinguished characteristics of a parasite

2 *Large Intra corpuscular Forms* (Fig 3) — They appear as more or less extensive areas of blue protoplasm, with one or more distinct red areas Pigment may be seen scattered over the parasite These large forms are generally simple tertian or quartan parasites

3 *Crescent and Crescent derived Bodies*—These are most definite bodies and readily recognized by the coarse pigment granules centrally situated The presence of this pigment should absolutely

preclude the possibility of mistaking distorted red cells crescentic in shape or a crescentic mass of platelets for parasites. In neither of these is there a definite central pigment mass nor should a foreign body be mistaken for a crescent. Moreover crescents again have quite definite outlines, and shew a red stained central portion and blue extremities.



Fig. 3A. Pigmented Large Mononuclear Leucocytes

The same criteria apply to the spherical form of the crescent.

4. *Pigmented Leucocytes* (Fig. 3A).—Large leucocytes with a large nucleus. Pigment (melanin) may occur scattered about the periphery of the cell or in little clumps or even in very fine powdery grains. The pigment is brownish black in colour. Slate pigment may be seen in epithelium scales or free in the plasma but the definite position of the pigment in the protoplasm of the leucocyte characterizes melanin.

Appearances in a Fresh Specimen

1. *Rings.*—The very small forms of these are characteristic of malignant tertian infection. They measure about one seventh the diameter of a red cell. A ring is characterized by its rather opaque white look, its very definite contour, and

and in a moment *three or four or more pale, long* processes are emitted. The red cells all around are put in motion by their violence and it may be only after a time when the activity has grown less that the flagella are actually seen. Nodosities will be observed in the flagella, and occasionally a speck of pigment at their extreme end. The flagella after a time break off, but they have only once by MacCallum, been seen penetrating the female gamete.

Under certain unknown conditions the crescents do not become spherical and eventually flagellate, but remain as crescents.

Breathing on the slide, adding a trace of water etc. have been recommended to produce the change more certainly but it is probable that the real cause lies in the state of development of the crescent for certain observations e.g., those of Major Buchanan I.M.S. shew that there is a certain time after the fever when a maximum number of gametes flagellate.

To Stain Flagellated Bodies

1. When flagellation is observed the cover glass is removed as carefully as possible and slide and coverglass are then *fixed* and *stained* with Romanowsky.

2. A number of rather thick drops of blood are placed on a series of slides. These are inverted over rectangular holes cut in blotting paper moistened with water and spread on a sheet of glass. A series of moist chambers is thus made. A dozen or more films are made and each one is removed at intervals of five minutes, dried

(spreading out somewhat if necessary) fixed and stained with ROMANOWSKY or dry the thick film, decolourize with water stain with ROMANOWSKY (without fixing) as in Professor Ross method of making thick films



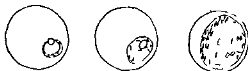




Fig. 4. The three Species of Malarial Parasites

To Determine the Species of Parasite Present

Three forms are recognized – simple tertian malignant tertian and quartan. The malignant tertian can as we shall see produce a quotidian

temperature with only a single generation of parasites Whether or no there is a true quotidian parasite one or more, is extremely doubtful

1 Minute rings one sixth to one seventh the diameter of a red cell, showing the signet ring shape, are characteristic of malignant tertian (Fig 4)

2 Large rings — If, when the temperature of the patient is still high the rings are of considerable size one fourth to one third of the red cell they are probably simple tertian or quartan If on the contrary the temperature is low and the febrile attack finished these forms correspond to fully developed malignant tertian parasites

The large malignant tertian rings have a characteristic appearance They are *oval* with a thicker layer of protoplasm (blue) opposite the nucleus (red)

3 Large forms with considerable blue protoplasm and with pigment granules, are *probably* simple tertian or quartan

The tertian parasite is an irregular and flimsy looking body and the medium sizes may show several pseudopodia (Fig 4) Pigment is scattered throughout and is actively motile while the quartan parasite is oval or globular of compact appearance with darker, coarser pigment shewing but slow motion (Fig 4)

The *enlargement of the cell* in which the simple tertian lies is also very *characteristic*

In a well stained specimen we have the further characteristic differences

1 *Simple Tertian* — The cell is dotted all over with fine red granules (SCHÜFFNER'S dots) these cells strike the eye during the microscopic examination and are diagnostic (Fig 4)

2. *Malignant Tertian*.—In specimens deeply stained with ROMANOWSKY the malignant tertian parasite also produces changes in the red cell (Fig. 4). These consist of coarse dots or clefts especially around the parasite. They are few in number and are equally characteristic of this parasite. Their appearance is quite different from SCHÜFFNER'S dots.

MAURER recommends the following method of developing them:—

10 drops of methylene blue (stock solution) + 25 c.c. of tap water.
15 drops of eosin (stock solution) + 25 c.c. of water.

Mix and stain for five minutes, shaking actively the whole time.



Fig. 5. 1. *Quartan parasites, segmenting forms.* 2. *Simple Tertian.* 3. *Malignant Tertian.* 4. *Quotidian (after ZIEMANN).*

3. *Quartan*.—The red cell shows no altered staining characters, but it may appear even smaller than normal. The parasite is not irregular in shape, but compact, oval or globular. (In the fresh specimen the parasite is very refractile and has a peculiar opaque look.) (Fig. 4.)

The finding of crescents, is, of course, diagnostic of malignant tertian, but the possibility of a double infection *e g*, simple and malignant tertian must be borne in mind

We have so far described the forms generally encountered during a febrile attack and the means of making a diagnosis but it is necessary to consider other forms *e g* the sporulating forms and more especially the gametes

Sporulating Forms —Besides the sporulating forms or segmenting forms we can recognize also the presegmenting forms in which the pigment begins to collect into a single mass and the chromatin gets split into a number of fragments These are even commoner than the final or sporulating forms in which the segments or spores are arranged around a central mass, though frequently here also the appearances do not correspond with the diagrammatic exactitude of the text books The segmenting and presegmenting forms are best seen in a case of regular quartan

Quartan Sporulating Forms —The pigment is placed centrally or often laterally, and grouped around it can be seen several six to eight chromatin masses In the presegmenting forms the pigment has not yet condensed into a single block, and the distribution of the chromatin masses is still irregular In fresh preparations the typical daisy forms can be clearly seen (Fig 5)

Simple Tertian Sporulating Forms —Here the whole parasite mass is larger, and fifteen or more chromatin segments can be distinguished (Fig 5)

Malignant Tertian Sporulating Forms — Rarely seen in the circulation There are eight to ten chromatin masses (Fig 5)

Gametes

Simple Tertian.—The young forms which under certain unknown conditions also appear in the circulation are characterized by the fact that the chromatin appears in the centre of the vacuolic area (Ruge) while in the asexual forms (schizonts) it is applied laterally

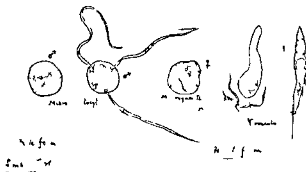


Fig 6 Male and Female Gametes (after [illegible])

The full grown gametes are much more easily distinguished The female gamete (♀) is characterized by the possession of much protoplasmic matter staining deep blue with Romanowsky and little chromatin in the ♀ the chromatin is laterally placed and is generally surrounded by a thin vacuolic area the pigment is black in colour and is irregularly scattered over the whole protoplasm (Fig 6)

The male gamete (♂) The chromatin is more voluminous than in the female it is of a looser texture that of the ♀ being compact the chromatin is centrally placed or extends in a broad

only very rarely indeed in the polymorphonuclear forms As a rule, a pigmented large mononuclear (Fig 3) is crowded with granules of pigment, the presence of only a few grains, or a single granular clump is exceptional The appearance of the clearly defined yellowish brown or black pigment granules in the clear protoplasm is so characteristic, that no doubt ought to exist It should be remembered however, that in dirty films specks of dirt may be over a leucocyte, and so resemble pigment In this case, similar specks will be found lying free The occurrence of malarial pigment free in the blood has never been seen by us

Leucocytic Variation —Often in cases where pigmented leucocytes are difficult to find there is a very obvious increase in the percentage of the *large mononuclear leucocytes* This change, which is usually very pronounced in the apyretic periods of an attack of malaria, is, however, most frequently absent during pyretic periods If, during a period of low temperature this change is not found there is a *strong presumption that the case is not malarial* If the blood be taken at the height of the fever a negative result does not exclude malaria and a further examination should be undertaken if possible during an apyretic period In some cases the change can be detected even during the pyretic periods but in these it is always more marked in the apyretic In some cases during the course of the fever, no such change occurs but appears immediately the temperature subsides and diminishes as convalescence proceeds Perhaps the cases where this test is of the greatest value are those where the patient has

already been treated with quinine and one can scarcely hope even if the disease be malaria to find parasites in the blood

To Make a Differential Count of the Leucocytes

Large films are necessary especially in malaria where during the apyretic period there is a distinct diminution in the *total* number of the white cells It is important in making films for leucocyte counting that the margins and terminal points of the film be regular and so in a convenient position for examination (Fig 1) The margin of the film is focussed and passed beneath the objective By passing along one half or the whole of the margin of the film the great majority of the leucocytes in the film are seen In order to obtain accurate results one thousand leucocytes should be counted but a count of three or four hundred is generally sufficient for diagnostic purposes Counts of a smaller number of leucocytes are valueless as too great variations will occur

As a leucocyte is seen it is marked under the heading large mononuclear intermediate transitional small mononuclear polynuclear eosinophil as the case may be As many as ten to twenty or more are mentally noted before making each record in its column

From the results obtained by blood counts of a considerable number of Europeans living in the tropics we found that an increase beyond fifteen per cent of the large mononuclear forms is proof of an actual or recent malarial infection whereas

with a value of twenty per cent it is almost always possible, by long search, to find an occasional parasite or pigmented leucocyte A value of over twenty per cent probably implies actual infection at the time of observation

The Normal leucocyte values are —

Polymorphonuclear leucocytes,		65 70	per cent
Large mononuclear	”	4 10	,
Intermediate	”		
Small mononuclear or lymphocytes	,	20 25	,
Eosinophil	”	2 4	,

Other forms of leucocytes, *e g*, mast cells, are always in extremely small numbers in health (0 5 per cent)

Chapter I

THE PARASITE IN THE TISSUES

Tissues may be readily examined for the presence of parasites or pigment in the following way —Place a minute portion of the tissue on a slide and with the end of another slide spread it out as evenly and thinly as possible Dry fix and stain in the same way as a blood film Parasites if present are in this way much more easily and clearly seen than in sections Spleen pulp bone marrow kidney liver etc give beautiful results and in the same way any secretion or fluid can be examined For certain tissues *e g* bone marrow it is advisable to fix in—

Absolute alcohol 1 part
Ether 2 parts

in order to dissolve out the fat present

To Prepare Tissues

In preparing tissues for examination —

1 Use as small pieces as possible *i e* at most five mm thick

2 Use plenty of the fixing and hardening fluid

3 See that the separate pieces do not cohere to one another Place some cotton wool on the bottom of the vessel

Corked collecting tubes will be found most convenient and will hold ample material The large masses of tissues sometimes sent home are of far less value to the pathologist than much smaller pieces well fixed and hardened Always put a label *in* the fluid, with the data written on it in pencil, as well as the outside label

Fixing

1 Alcohol is on the whole the most useful fixing fluid Small pieces of tissue should be put directly into absolute alcohol Larger pieces should be placed in ninety five per cent alcohol for two or three days and then for twenty four hours in absolute alcohol Intestine should be spread on filter paper, as also nerves or other tissue which it is desired to keep flat When removing the tissue from the paper, care should be taken that no fibres of the paper adhere as they may prevent the proper cutting of sections

For other modes of fixing *vide* appendix

To Store Tissues

Keep tissues in diluted alcohol (seventy five per cent about) If kept long in absolute alcohol many tissues become very hard

To Embed Tissues for Section Cutting

Except for very special reasons, embedding in paraffin should always be the method employed Very general misconceptions exist as to the *time*

and trouble necessary to prepare tissues in this way It may be pointed out —

1 That the times usually given for immersion in paraffin and other reagents are unnecessarily long

2 That the use of two paraffins for embedding, a soft and a hard is an unnecessary and even harmful procedure

3 That an elaborate apparatus for the paraffin bath is unnecessary (*vide* later)

4 By using flat and very thin pieces of material sections of considerable area may be obtained in a minimum of time It is necessary to cut thin slices of the raw material (1 mm or less in thickness) and place these upon a small piece of paper before placing in the alcohol to harden The paper keeps the slab from becoming distorted and enables one to cut sections of the full area of the slab say two fifths inch square

5 By placing minute pieces of tissue (in slabs on paper if a section of some size is needed) directly into absolute alcohol fixing hardening and dehydration can be accomplished within half an hour

NECESSARY APPARATUS FOR PARAFFIN SECTIONS

1 *Cambridge Rocking Microtome* The ordinary form is all that is necessary costing about five pounds It is convenient to have a ball and socket adjustable holder which enables one to change the angle of the block without remelting the paraffin

2 *Razors* These may be hollow ground on one side or on both to a varying depth For

general use a moderately hollow ground razor is used. Examine the edge under a low power to see if any notches exist; if so they must be ground out on a hone. A 'water of Ayr' stone as long as possible, should be used and kept absolutely free from grit during use. The stone should be soft, capable of being scratched with a pin, and as a lubricant water or filtered kerosene oil may be used. After honing the razor should be stropped. On one side of the strop a *minimum* amount of razor paste should be rubbed in and the leather side should be kept scrupulously clean and dry. If the razor is hollow ground on one side only, it should only be honed on this side.

Examined under the microscope the edge should now present a clear, sharp line. It may be tested on a thin hair, which it should easily cut.

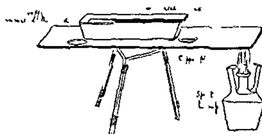


Fig 8. Simple Embedding Apparatus

3. *Embedding Apparatus.*—A slab of metal (copper) 12 × 3 × ¼ inches. Heat this at one end, and place the vessel containing the paraffin at a point on the slab where the paraffin is just

kept melted This is the temperature for embedding. This simple device serves all the purposes of an elaborate paraffin oven (Fig 8)

4 *Alcohol* — Absolute alcohol in the tropics has absorbed a good deal of water and it is necessary to dehydrate it

Heat crystals of $CuSO_4$ till a white mass is formed Allow to cool and place in a tall bottle of alcohol Allow to settle and decant off alcohol as required Add fresh anhydrous $CuSO_4$ if a marked blue tint develops or tie up the anhydrous copper sulphate in a muslin bag and place in the alcohol pot

Gelatine may be used to dehydrate alcohol it must previously be washed free from salts by soaking in water

5 *Xylol* — Xylol is the most generally satisfactory agent for displacing the alcohol and allowing the paraffin to permeate the tissue Chloroform wood naphtha turpentine oil of cloves and other substances may be used

6 *Paraffin* For use in the tropics paraffin melting at sixty degrees will scarcely be found too hard At high altitudes a softer will be required, and the right degree of softness must be determined and produced by mixtures of paraffin melting at 60 C and paraffin of lower melting point say 50 C, such as is suitable for use in temperate climates

To obtain paraffin suitable for use in a given temperature place a block of paraffin in holder and cut thin sections

(*a*) If the sections curl very much the paraffin is too hard

(*b*) If the sections formed are forced together (telescoped) the paraffin is too soft

(*c*) A certain amount of crinkling is usual with thin sections and can subsequently be got rid of before mounting

To Embed Tissues

1 *Alcohol* —Several changes If soft tissues *e g* liver spleen time is unimportant so long as dehydration is complete If fibrous organs the least possible time that will ensure dehydration (using thin slabs) Fibrous tissue becomes excessively hard if left too long in alcohol xylol or paraffin thus skin and connective tissue require great care in preparation

2 *Xylol* — Ten to twenty minutes When the tissues become transparent they are ready and should be transferred without delay to melted paraffin

3 *Paraffin* — Ten to thirty minutes If a tin trough be used the tissues should not be allowed to rest upon the bottom of the trough but be supported upon a strip of paper kept in place by folding the ends over the edge of the trough A watch glass generally suffices

4 Prepare a block for cutting by one of the following methods —

(i) If the piece of tissue be small, smear a watch glass with glycerine fill with melted paraffin and add the piece of tissue picked out of the bath with forceps warmed by passing through the flame

(ii) Fold a piece of paper so that by folding a trough of required size is made If an extra

length of paper be left at each end of the trough it can be folded down and holds the rest in position. Fill with freshly melted paraffin and add the piece of tissue picked up with warmed forceps.

(iii) Use metal pieces supplied with most microtomes upon a slab of glass.

The following points should be borne in mind —

(i) Fresh paraffin should be melted for the block, as paraffin frequently melted or kept melted for long periods does not form so uniform a mass when cooled as freshly melted paraffin.

(ii) The more rapidly the paraffin is cooled the more uniform is the resulting mass. It is well therefore, as soon as a well marked surface crust appears, to plunge the watch glass or trough into cold water.

When cold cut out a square block with the tissue arranged in the position required for the sections.

5. *Cut sections.*
Note (i) The angle the knife is placed at is important and must be found by experience.

(ii) It is well to use pads of paper to protect the edge of the razor where it presses against the iron of the microtome.

(iii) To cut in ribbons the top and bottom edges of the block must be parallel and horizontal. It is well to dip the block in soft paraffin or merely to smear the top and bottom surfaces of the block with soft melted paraffin.

(iv) Cut as thin sections as will remain intact.

To Stain and Mount Tissues

1. If the sections are crumpled float them upon water just hot enough not to melt the paraffin. They will become quite flat. Float the flattened sections on to a clean slide. Remove excess of water and firmly press a piece of filter or blotting paper over the section. Thoroughly dry by holding a few minutes over the flame (care being taken not to melt the paraffin) or by placing for twenty hours in a dessicator or warm oven. No further fixative is generally needed. If necessary the slide may previously be smeared with egg albumen fixative. It must in this case, be dipped rapidly into the water and quickly withdrawn. (Appendix.)

2. If the sections are flat they may be placed directly upon a slide slightly smeared with fixative. In this case celloidin in oil of cloves is the best fixative. (Appendix.)

3. Hold the slide or coverglass with the section over the flame till the paraffin melts. Dissolve off the paraffin with xylol, and then drop alcohol over the section. Place the slide or cover glass in water.

4. *Stain.*—The best stains for general use are:—

(i) Haematein purissimus saturated solution in 70 per cent alcohol 10 cc
Alum solution (alum 50 grammes, water 1 000 cc.) 50 cc

Stain for five to twenty minutes according to the depth of colour of the sections.

(ii) Methylene blue, or gentian violet

(iii) Counterstain if desired with watery eosin. For the detection of pigment it is well to stain a section faintly with eosin alone.

5. Pass through alcohol, oil of cloves, to Canada balsam. In hot moist climates, the cold produced by the evaporation of the alcohol causes dew to be deposited upon the slide. When the xylol or oil of cloves is added this produces a troublesome milkiness and may spoil the section. To avoid this all excess should be rapidly wiped up after the use of alcohol and the oil of cloves added as quickly as possible.

Chapter VI

THE MALARIAL PARASITE

Life History

Among the groups into which the protozoa are divided we find such well known classes as the Sarkodina *e g*, Amoeba Coli the Mastigophora possessing flagella *e g* Trypanosomes, and the Sporozoa It is these last that chiefly concern us The Sporozoa include such orders as the Gregarines (*e g* monocystis in the testes of the earth worm) and the Haemosporidia (which include the malaria parasites of man, and blood parasites of birds etc) There is a close relationship between the coccidia and the haemosporidia (malaria parasite) the delevopmental cycles of the two being almost identical The developmental cycle in the blood (the febrile cycle) of the malaria parasites was first demonstrated by Golgi the further cycle in the mosquito by Ross The cycle of Golgi is the asexual cycle, producing auto infection of the patient the cycle of Ross is the sexual cycle producing a new infection in a healthy subject

The sexual cycle it has been thought, commences in the blood when the conditions are unfavourable for the continuance of the asexual cycle and in fact has been taken as a sign that the

patient has already developed an immunity against the fever producing young parasites (spores). Thus it is well known that in malignant tertian the sexual forms gametes or crescents first appear about a week to ten days after the first febrile attack. If this view be true then it follows that the gametes develop from forms already present in the system viz. the asexual forms and possibly the divergence into sexual forms takes place from the youngest form of the parasite i.e. the spore. But it is possible that the divergence takes place at a stage previous to the youngest form of parasite i.e. at a stage immediately succeeding the entry *of sporozoits into the blood so that we have from* the first asexual and sexual forms present. Sexual development has been supposed to proceed mainly *in the internal organs e.g. bone marrow* but it is being gradually recognized that young forms of gametes are also found in the circulation, the characters of these have already been noted (p. 51). Let us suppose however that we are now dealing with fully developed gametes in the blood. We shall proceed to describe the further changes undergone in the mosquito. The male cell is as we have seen called the mikrogametocyte, the female cell the makrogamete. These we can distinguish in the blood. Further flagellation can be observed i.e. the protrusion of so-called flagella i.e. mikrogametes or spermatozoa. These flagella break off and fertilize the female cell the makrogamete, a process which has been con[illegible] in halteridium [illegible] but [illegible] in common.

This fertilized female cell is sometimes known as a Zygote though it is more convenient to reserve the term for a little later stage and to call the stage

the Vermiculus or Ookinet (Figs 6 and 9) Both these terms are suitable ones, for the first describes the fact that the fertilized female becomes worm like in shape and the second that the fertilized

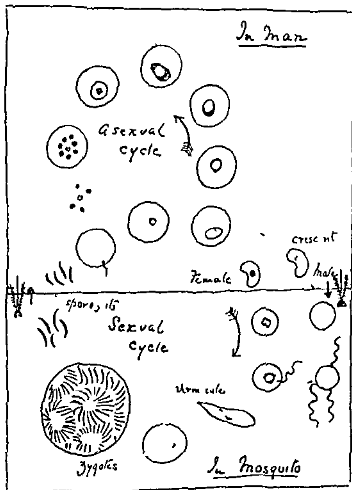


Fig 9 Life Cycle of the Malaria Parasite in Man and the Mosquito

egg moves. The vermiculus stage can be seen on the slide in the case of halteridium but in the case of malaria parasites only by taking the blood from the stomach of the mosquito after a suitable lapse of time. The vermiculus now finds its way through the epithelium of the stomach and then lies in the external muscular layers as a spherical or ovoid body, the zygote. A kind of capsule is formed around it by these tissues and so at this stage it is also called the *Oocyst*. Growth proceeds and signs of division into several masses appear in the protoplasm. This stage is termed a medium zygote or sporoblast. Then we reach the stage of large zygote (or sporoblast) and by this time the masses of the sporoblast have undergone division into a number of fine curved thread-like bodies, the sporozoits, so that eventually the large cyst is almost entirely filled with sporozoits. The capsule of the cyst eventually ruptures and the sporozoits pass from the tissues of the stomach to the thorax, being found at first amidst the muscles, but eventually all collected in the salivary glands. From here they are injected into the blood by the mosquito and they then attach themselves to and penetrate the red cells (as has been actually observed under the microscope by SCHAUDINN) producing a new infection.

We may briefly summarize these various steps :—

1. Mikrogametocyte and makrogamete in blood.

2. Development of mikrogametes = flagellation.

3. Fertilization of the makrogamete = ovum or copula.

4 Vermiculus or ookinet
5 Zygote or oocyst
6 Medium or large zygote = sporoblast
7 Sporozoits

The sexual cycle is known also as sporogony or amphigony while the asexual cycle is known as schizogony or monogony These two cycles and their relation to one another are shewn in the figure (Fig 9)

Further there is a certain amount of evidence to shew that a gamete in the blood can undergo a kind of retrogressive development, and give rise directly to young parasites (*i e*, schizonts) If this is so it would explain the supposed function of old attributed to crescents (gametes) of producing relapses

Chapter VII

MOSQUITOES

Mosquitoes belong to the order of Diptera or true flies which are characterized by

1. A single pair of membranous wings
2. Suctorial mouth
3. Complete metamorphosis

In all mosquitoes except the genera Corethra and Mochlonyx there is a long piercing proboscis which is characteristic of the *Culicidae*. Mosquitoes usually are about five mm in length but certain species e.g. Megarrhina are much larger.

Flies which may be mistaken for mosquitoes are —

1. *Chironomus* — The chironomidae or midges are more delicate in structure than most mosquitoes and are often bright green or pale yellow in



Fig. 1 Chironomus

colour. They do not possess the characteristic proboscis of mosquitoes. The veins of their wings are more complex and are *quite devoid of*

scales The absence of scales upon the veins of the wings at once distinguishes these from true mosquitoes (Fig 10)

Enormous numbers of Chironomidae are found near water especially sedgy rivers and swamps They are attracted by light, and are constantly seen around lamps and candles, a position in which true mosquitoes are scarcely ever found

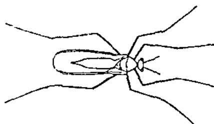


Fig 11 *Trichocera*

2 *Trichocera* —Some small Tipulidae often possess a considerable superficial resemblance to mosquitoes as for example the winter gnat (trichocera) When at rest their bodies lie parallel with the surface and upon it They have no distinct proboscis (Fig 11)

3 *Cecidomyidae* or gall midges —These have a simple wing venation and there are no forked cells In most species the wings and bodies are hairy not scaled

4 *Rhyphidae* —These are readily distinguished on a close examination Their wings are spotted

Other flies than mosquitoes suck blood

1 *Simulidae* or sand flies (sometimes also called midges) —These are minute flies which

suck blood voraciously They have a short and stout proboscis The salivary glands are very large in proportion to the size of the fly and the bite is as severe as that of a mosquito The males are harmless (Fig 12)

The larvae of the Simuliidae are aquatic, cylindrical in shape and live in the stems of water plants The imago hatches beneath the water

2 *Phlebotomus* (family Psychodidae or owl midges)—Small fluffy looking flies which suck blood readily They are most readily detected after feeding when the abdomen is swollen with blood They have very hairy wings and body and a short powerful proboscis (Fig 12)

3 *Tabanidae* Large flies of heavy build (appendix p iv)



Fig 12 Sand Fly (left) Owl Midge (right)

4 *Hippoboscidae* Large flies of heavy build inflicting a severe bite They do not lay eggs the larval and pupal stage going on in the mother (appendix p vi)

5 —Tsetse flies (*Glossina*) etc (*vide* p 353)

scales The absence of scales upon the veins of the wings at once distinguishes these from true mosquitoes (Fig 10)

Enormous numbers of Chironomidae are found near water, especially sedgy rivers and swamps. They are attracted by light, and are constantly seen around lamps and candles, a position in which true mosquitoes are scarcely ever found

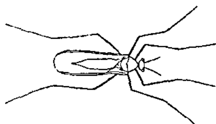


Fig 11 *Trichocera*

2 *Trichocera* —Some small Tipulidae often possess a considerable superficial resemblance to mosquitoes as for example the winter gnat (trichocera) When at rest their bodies lie parallel with the surface and upon it They have no distinct proboscis (Fig 11)

3 *Cecidomyidae* or gall midges —These have a simple wing venation and there are no forked cells In most species the wings and bodies are hairy not scaled

4 *Rhyphidae* —These are readily distinguished on a close examination Their wings are spotted

Other flies than mosquitoes suck blood

1 *Simulidae* or sand flies (sometimes also called midges) —These are minute flies which

suck blood voraciously. They have a short and stout proboscis. The salivary glands are very large in proportion to the size of the fly and the bite is as severe as that of a mosquito. The males are harmless (Fig 12).

The larvae of the Simulidae are aquatic, cylindrical in shape and live in the stems of water plants. The imago hatches beneath the water.

2. *Phlebotomus* (family Psychodidae or owl midges).—Small fluffy looking flies which suck blood readily. They are most readily detected after feeding when the abdomen is swollen with blood. They have very hairy wings and body and a short powerful proboscis (Fig 12).

3. *Tabanidae*.—Large flies of heavy build (appendix p iv).



Fig 1 Sand Fly (left) Owl Midge (right)

4. *Hippoboscidae*.—Large flies of heavy build inflicting a severe bite. They do not lay eggs, the larval and pupal stage going on in the mother (appendix p vi).

5.—Tsetse flies (*Glossina*) etc (*vide* p 353).

Life History of the Mosquito

In common with all other insects shewing complete metamorphosis, the mosquito passes through four stages —

The egg
The larva
The nymph
The imago

The Imago —The imago is the well known winged insect The emergence of the imago may be seen on the surface of almost any collection of

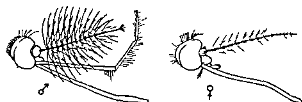


Fig 13 Heads of Male (♂) and Female (♀) Culex

foul water Shortly after hatching the insect may be seen resting quietly upon the surface of the water and does not fly away when disturbed or only very feebly For some considerable time after hatching (twenty four hours) the insects refuse to feed

In the imago there are marked differences between the male and the female insect

The Male —In the male the antennae are markedly plumose The palps also are long and hairy The effect is to make the head of the male mosquito very conspicuous (Fig 13)

The male mosquito with the exception of certain species does not feed upon blood and the proboscis is only used to suck in vegetable juices The male of *Stegomyia* mosquitoes however sucks blood like the female

The Female—In the female the antennae are inconspicuous and have only short lateral hairs The palps are also less conspicuous than in the male (Fig 13)

The female feeds upon blood and is frequently seen with the stomach distended with blood more or less digested

The female is also seen with the abdomen more or less swollen with the greatly enlarged ovaries which give a whitish and opaque colour to the mosquito and often make the insect much more conspicuous in its flight than it otherwise would be

The commonest species of mosquitoes belong largely to the following genera or closely related forms —

1 *Anophelina* (sub family)
2 *Culex*
3 *Stegomyia*
4 *Taeniorhynchus* and *Panoplites*
5 *Uranotaenia*

The sub family *Anophelina* is in many ways the most distinct of these groups Not only are the adult insects highly characteristic in appearance but the ovum and larva are quite unlike those of any other genus One indeed can recognize the *Anophelina* at a glance merely by their characteristic general appearance once the peculiarities of this genus are known

upon a wall its body projects so as to form a distinct angle with it. In some cases the angle assumed is almost a right angle. In the case of almost all other mosquitoes the body is held either parallel with the wall or what is more frequent the tail approaches the wall giving the insect a hunchbacked appearance. This difference is readily seen by any careful observer, and is a practical and useful distinction. A characteristic of *Anopheles* is that it rests by preference on the first two pairs of legs only, and keeps the last pair *stretched out stiff* and *straight* or they slowly oscillate to and fro. Many mosquitoes wave the hind legs notably *Stegomyia* but they are held with the tarsi curved backwards.

The exact attitude adopted depends upon the species and the situation, whether a vertical or horizontal surface on which the *Anopheles* is resting. One very common species *(M culicifacies)* at least when sitting on a wall, looks exactly like a small brown *Culex* since it holds its body parallel with the wall as a *Culex* does.

Culex.—Mosquitoes of the genus *Culex* are many of them brown mosquitoes of sober hue, *e g* the common house *Culex*, *C fatigans* which is uniformly brown without markings. The genus however contains a very large number of species. In *Culex* mosquitoes the attitude when resting is 'hunchback.'

Stegomyia.—The genus *Stegomyia* is of the greatest interest and importance since it is this form which is concerned in the transmission of yellow fever (*Stegomyia fasciata*).

These mosquitoes are generally black and white with banded legs and abdomen and spots

on the thorax. They are found in houses and are most troublesome mosquitoes from their habit of feeding in the day, and their great alertness and persistence. *Stegomyia* are also very common in woods and forests.

Both male and female suck blood.

Taeniorhynchus (for exact distinctions *vide* later p. 179).

Mosquitoes of the genus *Taeniorhynchus* are most frequently banded yellowish brown.

They occur especially in the jungle near swamps, river margins, etc., and in Africa at least may aptly be termed "bush" mosquitoes.

Panoplites.—*Panoplites* are closely related to *Taeniorhynchus*. They are heavily scaled insects, almost floury in appearance. They occur especially near swamps, etc.

CAPTURE OF MOSQUITOES AND FLIES

1. Place a lamp upon a sheet of white paper and note the insects which are attracted by the light. Note insects belonging to the orders Lepidoptera (moths), Hemiptera (aphides, green flies, etc.), Heteroptera (plant bugs), Neuroptera (caddis flies, stone flies, white ants). Pick out any mosquito-like flies. They will probably belong to the Chironomidae. Note the absence of proboscis, the delicate transparent structure. Note the plumed head of the male (as in the true mosquito) and the less conspicuous antennae of the female. Examine the wings under a strong lens or a low power of the microscope and note that the wing veins are bare and do not carry any scales. Note that true mosquitoes are not seen around the lamp.

2 Examine, with a light some wall which has been only dimly illuminated by the lamp *i e* some wall at the distance of several yards, and note true mosquitoes resting upon this Capture several of these by placing a tumbler over them and kill them by puffing in a little tobacco smoke Observe that they have a distinct proboscis Observe the plumed male and the female without plumes Examine the wings under a strong lens or low power objective and note the scales attached to the wing veins

The specimens caught will probably be specimens of *Culex* If near a swamp or jungly place there may be *Taeniorhyncus, Panoplites* and possibly *Anopheles* Observe the hunchback attitude in the case of most of the mosquitoes caught *If an Anopheles should by chance be* caught note the striking difference in the general appearance the attitude and the spots on the wings

3 Observe in stuffy furnished rooms offices etc the presence of mosquitoes feeding actively during the day Capture some of these They will probably belong to the genus *Stegomyia* Note their extreme alertness Observe that they are black with white bands Note the habit of waving the hind legs and that the tarsi of these are kept curved Ascertain whether the males feed upon blood

4 Examine stables huts, outhouses in the early morning

LITERATURE

The Cambridge Natural History Diptera A most useful book for an introductory knowledge of a variety of winged life in the tropics and elsewhere

Chapter VIII

THE OVUM

Ova are minute bodies one mm… length When first laid they are whit… but become rapidly brown or black … on the surface of water and if submer…



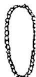


Fig … Eggs of … mosq…

hatch out Mosquito eggs may be … edge of water or on floating objects … water In the last case they have som…





Fig … Egg … and Egg of Cu…

separately upon the water, and has air cells which keep it afloat In the case of *Culex* and *Taeniorhynchus* hundreds of eggs are cemented together to form rafts each egg lying perpendicularly, with its larger end pointing downwards In *Culex*, the



Fig 18 Egg Raft and Eggs of Taeniorhynchus

egg rafts are broad and roughly oval in shape (Fig 17) In *Taeniorhyncus* the egg raft is extraordinarily elongated resembling in shape, a racing skiff (Fig 18)

THE EXAMINATION OF OVA

Culex —Examine the surface of some semi putrid water for egg rafts of *Culex* Egg rafts can almost always be found on the surface of water containing macerating leaves fruit etc They are bodies of a blackish brown colour and are readily wafted about by the wind

1 Note that the raft is boat shaped measuring one fifth to one third inch in length and consists of two hundred to four hundred eggs

2 Note that the separate ova are smooth elongated bodies about seven to eight mm in length Note that there are no floats or other markings as in the case of *Anopheles* ova

3 Note that one end of the egg is thicker and blunter than the other and that to the thicker

end is attached a clear transparent globular body (the micropilar apparatus) Note that this body is readily detached, often leaving a spike like process projecting from the thicker end of the ovum

4 Note that the thicker end of the egg is placed downwards and lies in the water As certain by keeping the egg rafts upon water until the larvae hatch that the young larvae breaks through the lower end of the ovum

5 Make as many observations as possible upon the egg rafts e g time necessary for hatching of larvae amount of desiccation they will withstand

Anopheles —The ova of *Anopheles* are difficult to detect in nature but may be seen by the aid of a lens on the margins of small pools where larvae abound They are about 0 7 to 1 0 mm long

Examination of Anopheles Ova —

1 Confine some female *Anopheles* as described on p 120 Endeavour to choose those in which the ovaries are nearly mature (p 97) Fifty to one hundred and fifty eggs are laid Remove the piece of paper upon which the ova have been deposited and place this upon a slide Examine with a low power in strong daylight and the mirror turned off

2 Observe the remarkable resemblance of the ova to little boats and the presence of the two beautiful oval air cells placed upon either side acting as floats (These are absent only in one species as yet described viz *M turkhudi*) Observe also the presence of a white frill or a mere ribbed rim around what would be the gunwale of the boat (Fig 54)

separately upon the water, and has air cells which keep it afloat. In the case of *Culex* and *Taeniorhynchus* hundreds of eggs are cemented together to form rafts, each egg lying perpendicularly, with its larger end pointing downwards. In *Culex* the

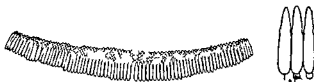


Fig. 18. Egg Raft and Eggs of Taeniorhynchus

egg rafts are broad and roughly oval in shape (Fig. 17). In *Taeniorhyncus* the egg raft is extraordinarily elongated, resembling in shape a racing skiff (Fig. 18).

The Examination of Ova

Culex.—Examine the surface of some semi-putrid water for egg rafts of *Culex*. Egg rafts can almost always be found on the surface of water containing macerating leaves, fruit, etc. They are bodies of a blackish brown colour and are readily wafted about by the wind.

1. Note that the raft is boat shaped, measuring one fifth to one third inch in length, and consists of two hundred to four hundred eggs.

2. Note that the separate ova are smooth elongated bodies, about seven to eight mm in length. Note that there are no floats or other markings as in the case of *Anopheles* ova.

3. Note that one end of the egg is thicker and blunter than the other and that to the thicker

end is attached a clear transparent globular body (the micropilar apparatus) Note that this body is readily detached often leaving a spile like process projecting from the thicker end of the ovum

4 Note that the thicker end of the egg is placed downwards and lies in the water As certain by keeping the egg rafts upon water until the larvae hatch that the young larvae breaks through the lower end of the ovum

5 Make as many observations as possible upon the egg rafts *e g* time necessary for hatching of larvae amount of desiccation they will with stand

Anopheles —The ova of *Anophele* are difficult to detect in nature but may be seen by the aid of a lens on the margins of small pools where larvae abound They are about 0 7 to 1 0 mm long

Examination of Anopheles Ova —

1 Confine some female *Anopheles* as described on p 1_0 Endeavour to choose those in which the ovaries are nearly mature (p 97) Fifty to one hundred and fifty eggs are laid Remove the piece of paper upon which the ova have been deposited and place this upon a slide Examine with a low power in strong daylight and the mirror turned off

2 Observe the remarkable resemblance of the ova to little boats and the presence of the two beautiful oval air cells placed upon either side acting as floats (These are absent only in one species as yet described viz *M turkhudi*) Observe also the presence of a white frill or a mere ribbed rim around what would be the gunwale of the boat (Fig 34)

3 Observe that one end of the ovum is always stouter than the other The stout end contains the head of the embryo and is the head from which the young larva escapes Note also that when *Anopheles* eggs are seen at the side of vessels drawn up by capillarity the thick end is at the bottom Examine the surface of the water remaining in the hollow stopper or receptacle and observe that the ova of *Anopheles* are laid singly without any cement substance and float singly or touching one another on the water

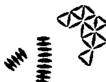


Fig 19 *Pattern formed by Eggs of Anopheles*

4 Observe star shaped patterns formed by some species or the arrangement in parallel groups assumed by the ova of others (Fig 19) Note that this arrangement is dependent on physical causes (shape of the egg, etc) and not on the fact that the eggs are laid in such positions This is readily done by stirring up a number of *Anopheles* ova on water and noting how they tend to form groups in triangles and star shapes

5 Ascertain that *Anopheles* ova when first laid are white but rapidly darken and become black Observe that *Anopheles* ova are very often laid in heaped up masses which eventually become dispersed by waves etc Observe that the eggs then form patterns

6 Place some half dried mud in a flat dish and put this inside a piece of mosquito netting in which some *Anopheles* with ripe ovaries are placed Observe that ova are laid upon the mud

7 Preserve the mud for forty eight hours preventing it from becoming completely dry

At the end of forty eight hours or more remove a few ova to a dry slide and place under a low power Allow a drop of water to flow on to the ova Observe the escape within a minute or so of the young larvae and the fact that a cap like piece of the egg shell is pushed off

8 Observe that *Anopheles* kept in a dry test tube will occasionally lay their eggs on the side of the tube

9 Note the time when the eggs were laid and the time at which the larvae emerge This depends greatly on the temperature It may take two to three days

10 Remove *Anopheles* ova on paper and allow them to dry and note that after two or three days at the most they will not hatch when carefully placed on water

Stegomyia - Confine some gravid females of *Stegomyia* mosquitos

1 Note that in some species the eggs are laid singly and much resemble at first sight the ova of *Anopheles* Note that in others the eggs are laid in rafts (*S notoscripta*)

2 Note that they are irregularly oval thicker at one end than the other and have a corrugated surface in which are entangled numerous minute air bubbles

3 Examine the surface of water left exposed for several days in a tumbler etc Note if

Stegomyia mosquitoes have oviposited, the presence of eggs occurring singly or in parallel groups Note that the ova are larger than those of *Anopheles* and that they hatch into *Culex* like larvae (see *Stegomyia* larvae p 85)

Taeniorhyncus —Examine natural waters, especially small pools with a dense growth of alga, swamp pools irrigated land, etc for the egg rafts of *Taeniorhyncus*

1 Observe the extreme length and narrowness of the rafts Note also how small a portion of the raft is submerged

2 Observe that the ova are arranged as in *Culex* rafts *with the thicker end downwards* and that they are smooth and have a micropilar apparatus

3 Endeavour to obtain the ova of known species of *Taeniorhyncus* by confining gravid females *Note the shape of the rafts*

Panoplites — Observe that the eggs have a curious snout like projection, and that they are laid singly

Psorophora — The eggs are large, two mm long They occur in patterns like those of *Anopheles* The eggs are covered with minute prickly scales

Observations upon the ova of the different species of mosquitoes are very meagre and full and accurate descriptions of these are of great value (see Chap XVIII)

Chapter IX

THE LARVA AND NYMPH

The Larva

The larvae of mosquitoes, more especially of *Culex*, are well known objects. They can be seen by holding up to the light almost any specimen of water that has been left undisturbed for some days, but especially water which contains macerating leaves.

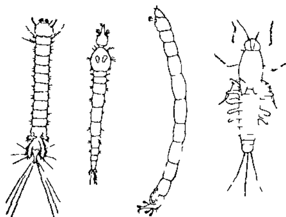


Fig. [illegible]. Larvae that may be mistaken for Mosquito Larvae

Larvae which may be mistaken for those of mosquitoes are

1 *Chironomus* —The larva of Chironomus is a red worm like creature (blood worms) and has no close resemblance to mosquito larvae (Fig 20)

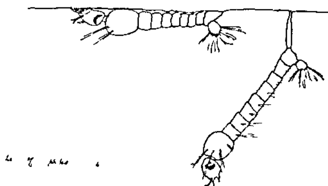


Fig 21 Larvae of Anopheles (left) and Culex (right)

2 *Ephemeridae* — The larvae of certain small Ephemeridae may at first glance be mistaken for mosquito larvae There is no real resemblance, and the triradiate tail of the ephemera larva at once distinguishes it (Fig 20)

3 The larva of dixa rather closely resembles the larva of *Anopheles*, though not other mosquito larvae (Fig 20)

EXAMINATION OF THE LARVA

Culex (Fig 21) —Obtain some *Culex* larvae from any source and place in a glass vessel

1 Observe the hanging attitude of the larva Note the angle it makes with the surface of the water, and how this varies in different species

Note, if the larva of *C concolor* is being examined that the position is nearly horizontal

2 Observe the large head the prominent eyes and projecting antennae

3 Note the long respiratory syphon arising from the eighth abdominal segment

Place a half grown larva under a coverglass and examine under one third inch objective

1 Make an accurate drawing of the antennae

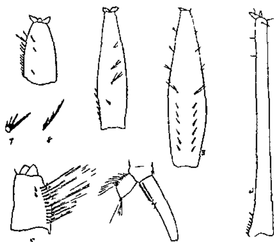


Fig Respiratory Syphons of Larvae
(1) *Stegomyia* ( ) *Culex* (3) *Culex with Large Syphon Tube* (4) *Taeniorhynchus* (5) *Cinnidal Larva (? C C ncolor)* (6) *Enormous Syphon Tube (one quarter scale of others genus undetermined)* (7) *Spine on Tube seen on the flat* (8) *Spine seen sideways*

2 Carefully observe the length and thickness of the respiratory syphon

3 Note the absence of palmate hairs

4 Determine whether the larvae examined are *Culex Stegomyia Taeniorhynchus* etc

straight alimentary canal Place in a drop of clean water and crush under a coverglass Note what organisms form the chief bulk of the food Note the presence of sand grains—unicellular plants and animals—short lengths of alga, diatoms, etc Also bacilli

Determine the common foods of several species of *Anopheles*

4 *Culex Larvae* — The larvae of the *Culicidae* with the exception of those of *Anopheles* and possibly some other genera are superficially much alike The conspicuous hairs and spines and even the complicated terminal segment, are very similar in the different genera There are however marked differences in some features on closer examination These differences are mainly to be found in the syphon tube, the antennae and mental plate, but to a less extent in other structures

Examine some Culex Larvae —Note differences in naked eye appearance note the long worm like *Stegomyia* larva and its wriggling mode of progression note the transparent and spiny appearance of some larvae (notably *Taeniorhyncus*) note that some larvae adopt a nearly horizontal attitude (*C concolor* and others) others a vertical attitude (*Stegomyia*) whilst the majority form a small angle with the vertical Examine larvae under a low objective Note the head with eyes large feeding brushes antennae and various hairs note the large bunches of hairs arising from the thorax and abdominal segments note the last segment bearing four large clear papillae and two systems of hairs note the penultimate segment which carries the syphon tube and some curious claw like spines

Note especially the following —

(i) The syphon tube
(ii) The antennae
(iii) The mouth parts
(iv) The anal papillae
(v) The hairs of the thorax and abdomen

The Syphon Tube — This is formed of a single cylindrical piece of chitin and contains the origin of the two main tracheae of the body. Note the small flap like pieces of chitin forming a closing apparatus at the extreme tip. Measure (by eye piece micrometer) the length and greatest breadth of the syphon tube; note that in different species and especially in different genera the syphon tube varies greatly in its measurement. By dividing the length by the breadth a figure may be obtained which is useful and may be termed the syphonic index number; note that in *Stegomyia* this number is about two. In *Culex* four to seven. In *Taeniorhynchus* as much as twelve in some cases. Draw accurately by measurement (eye piece micrometer) a number of syphon tubes of different *Culex* larvae. Note that marked variations in different species exist.

Note two rows of spines on the posterior aspect of the syphon tube starting from the base and extending a variable (in different species) distance up the syphon tube; note that they differ in number and length etc. in different species. The spines appear serrated or compound according to the angle they are viewed from and differences may be supposed to exist which depend upon this fact. In some species (certain carnivorous or cannibal larvae) a large fan of hairs project posteriorly in the medium line from the syphon

tube In certain species the syphon tube is of enormous size, and may attain to one third the length of the larva

The Antennae—Note in the case of most typical *Culex* larvae that the antennae are large conspicuous objects note a basal medial and terminal portion and a large bunch of feathered hairs arising at the junction of the two first named portions, note also large single and stout hairs from the more terminal portion note spines on the body of antenna

Examine the Antennae of various Larvae—Note in some cases that the antennae are more rudimentary (*Stegomyia Anopheles*) In the case of *Stegomyia* (as far as described) they are small and spineless, and possess *only a small hair* arising from a papilla which may be single or in three or four branches Make drawings (using eye piece micrometer) and note great variation in different genera and species

The Mouth Parts – Note the characters of the claw like mandibles and especially the exact character of the triangular *mental plate which* forms a conspicuous dark triangular body on the under surface of the head

Note that in different species the plate varies in appearance especially in the size and number of notches in its margin In some species the plate is like a shark's tooth in others it is comb like

The Anal Papillae—Note the tracheae ramifying in these the papillae being possibly gill like in function In most species they are pointed in others they are globose at the end

The Large Body Hairs.—These are long in some larvae, much shorter in others; their arrangement is very similar in the different larvae.

5. *Cannabalism of Larvae.*—Add some large *Culex* larvae to a small bottle containing some small larvae or *Anopheles* larvae. The *Anopheles* larvae or small *Culex* larvae will be devoured by the large forms.

6. Observe the occurrence in nature of the two forms *Culex* and *Anopheles*; also what *Culex* larvae are found living together.

7. *The Enemies of Larvae.*—Add small fish, waterbeetles and their larvae (Dytiscidae, Hydrophilidae), libellula larvae, corysca, nepa, tadpoles and other water animals respectively to a series of wide mouthed bottles containing equal numbers of larvae. Note the rate at which they are devoured, if at all. The carnivorous forms nepa, corysca, libellula rapidly devour larvae. Hydrophilidae beetles, tadpoles, etc., do not destroy larvae. Observe that some species of fish are much more active devourers of larvae than others. Note that weeds often protect larvae from being consumed by small fish.

8. Make experiments with different chemical and other bodies and note the absence or presence of culicidal power.

(a) Note that chemical bodies in solution kill only with difficulty as a rule, e.g. corrosive sublimate. Ammonia however (1 in 4,000 of water) will kill mature larvae according to WADDELL.

(b) Note that oils rapidly kill larvae by blocking the air tubes. Treat larvae by pouring a little olive oil upon the water. Stain with osmic acid and note globules of oil within the air tubes.

9 Add some paraffin oil to a small *Anopheles* pool; observe the presence next morning of dead female mosquitoes that have come to lay their eggs. Observe the effect of paraffin on different kinds of natural water and the great efficacy in some cases and futility in others.

10 Observe that pools covered with *Lemna* are very frequently, if not always, free from larvae. The action of the Lemna is said to be mechanical.

EXAMINATION OF OTHER LARVAE

Dixa.—In its movement along the surface of the water the larva of *Dixa* resembles *Anopheles* larvae, and this larva also rests horizontally just beneath the surface film.

In *Dixa* there is no globular thorax, and the whole larva is longer and thinner than an *Anopheles* larva (8 mm.). Moreover, *Dixa* larva only indents the surface film at the head and tail, there being no palmate hairs on any of the segments. *Dixa* larvae move very rapidly and have a habit of climbing above the surface of the water *and resting in a loop* with the head and tail downwards. When placed in a specimen tube it climbs up the side and becomes lodged in crevices in the cork.

It is found frequently in running water (Fig. 20).

Mochlonyx Larva.—Note absence of palmate hairs on dorsum and presence of respiratory syphon, absent in *Anopheles*. They are extremely voracious. They lie deep in the water.

Corethra Larva.—These are the so-called phantom larvae. They are extremely transparent and lie horizontally rather deep in the water. The head is smaller than in *Anopheles*. There is no air system communicating with the external air. They are extremely voracious. Add some *Corethra* larvae to a tumbler containing *Culex* larvae.

Stegomyia. The larva of *Stegomyia* is rather longer than that of *Culex*. When disturbed it exhibits a rather lashing movement like that of certain small aquatic worms. When at rest at the surface the attitude of the body is almost vertical.

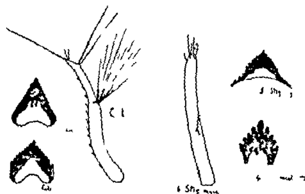


Fig. 3. Antennae and Mental Plates of Larvae.

The larva however spends a good deal of its time browsing at the bottom of the water and then lies for the most part horizontally.

The head is small in proportion to the rest of the body, and the thorax is less conspicuously marked off from the abdomen than in *Culex*.

The antennae resemble those of *Anopheles* larvae more than those of *Culex*. The large branched hair of *Culex* is represented by a short inconspicuous simple hair (or as many as three) projecting from the side of the antennae (Fig 23)

The syphon tube is characteristic, being very short and stout (Fig 22) only twice as long as broad whereas in *Culex* the syphon tube is four or more times as long as broad (Fig 22)

1 Examine domestic utensils, disused water pots, and tins containing water. Examine the water which collects in the axils of the banana leaves collections of water in tree stumps in the jungle. If larvae are present—

(i) Note the very short and broad spiracle tube and its dark colour. Compare with that of *Culex* and *Taeniorhynchus*

(ii) The larva is longer and more worm like than most mosquito larvae. The 'wriggling' motion is also very markedly shewn owing to the length of the body

Taeniorhynchus.—In natural waters especially shallow trickling water forming pools with a dense growth of spirogyra etc, swamp water and river margins, the larvae of *Taeniorhynchus* will be readily found

1 Note that the larva lies often embedded in the masses of green spirogyra or other thread like algae

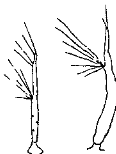
2 Note the great transparency of the larva and the frequency with which brilliantly green specimens are found

Under a low objective note the following which appear to be characteristic of this genus —

1 The enormous horn like and curved antennae (Fig. 23A)

2 The extreme length and slenderness of the syphon tube (Fig. 22)

Psorophora —The larvae are large half an inch in length They are extremely cannibalistic



Culex Fatigans *Taeniorhynchus*
Fig. 23A

Information upon the larvae of the different genera of mosquitoes is so meagre that in as many cases as possible the larvae of different species should be determined and systematic descriptions and drawings made of such important parts as the antennae syphon tube and such other parts as may be found to vary in the different larvae observed

NOTE —Further work on the syphon tube of larvae has shewn us that they are of *specific* as well as generic importance The extremely long and slender syphon tube is apparently not confined to *Taeniorhynchus* but mosquitoes having this type of tube have also [illegible] *lanlei prol* [illegible]

The Nymph

The nymphae of mosquitoes are extremely characteristic bodies. Wherever a number of fully developed larvae are found there will generally also be seen numbers of bulbous comma shaped creatures having a large globular body (head and thorax) and a small tail kept more or less tucked in beneath. When disturbed they dart downwards with great speed but very soon reappear at the surface.

Nymphs are not so easily seen in pools as larvae.

The differences in the nymphae of different genera of the *Culicidae* is not nearly so great as in the case of the larvae.

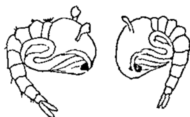


Fig 24. *Nymphae of Anopheles and Culex*

By keeping under observation a number of nymphae some will be seen to become less inclined for active movement and the abdominal segments (tail) may be extended horizontally. Soon after these changes the adult insect emerges through a crack in the chitin of the back of the

thorax The process as seen in *Anopheles* is very fully described by NUTTALL and SHIPLEY

EXAMINATION OF NYMPHAE

1 Note the effect of tapping the glass vessel and the rapidity with which the nymphs regain the surface

2 Observe that when first they appear the nymphs are light in colour but darken very considerably later

3 Note that just before the hatching of mosquitoes the nymph lies with the tail extended and that silvery marks may be seen due to air lying under the chitin

4 Observe the emergence of the imago

Examine the nymphs of *Anopheles*, *Culex*, *Taeniorhyncus*, etc and observe that to the naked eye they are very similar

1 Note that the nymphae of *Anopheles* lie less vertically in the water than those of *Culex*

2 Observe that the nymphs of *Anopheles* are more elongated antero posteriorly and compressed laterally than those of *Culex* and *Taeniorhyncus*

3 Observe the very large nymphs of some common species of *Taeniorhyncus* and the great length of air tubes which are directed straight forwards in a very characteristic manner

Place nymphae in drops of water on a slide and examine the air syphons Note—

1 In *Anopheles* the syphons have a square truncated end and are proportionally much shorter than in *Culex* and project from about the middle of the thorax (Figs 24 and 25)

Jou l Hyg I p 1 II

2 In *Culex* the syphons are long and narrow, and have a slit like opening, and project from the posterior portion of the thorax (Figs 24 and 25)

3 *In Stegomyia* the syphons are broadly triangular, and are characteristic Note the marked contrast in appearance to those of *Culex* (Fig 25A)

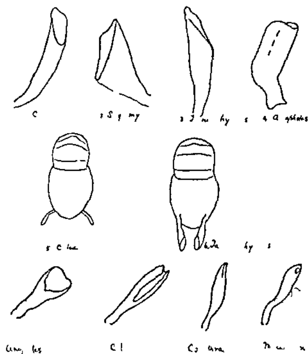


Fig 25 *Nymphal Syphon Tubes*

Examine the nymphs of *Corethra* and *Mochlonyx* when they are encountered

(*a*) Note in *Corethra* the pointed syphons and the straight tail (Figs 25 and 26)

(*b*) Note in *Mochlonyx* the *Culex* like nymph and the thin rounded and pointed syphons (Fig 25)

Examine the bottoms of pools of polluted water and note in the mud the brilliant red nymphs and larvae of *Chironomus* (Fig 26)

1 Observe that the *Chironomus* nymph has a large globular body (head and thorax) and bears a general resemblance to mosquito nymphs



Fig 5x Nymphal Syphon Tube of St gomyia

2 Note however the presence of the conspicuous white feathery gills which form tufts at the side of the head

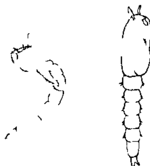


Fig 6 Nymphs of Chironomus and Corethra

3 Note that the *Chironomus* nymph and larvae do not rise to the surface to breathe as do those of mosquitoes

4 Note the curious rhythmic bending movement of the larvae and nymph of *Chironomus* which, when they are present in numbers, give the mud at the bottom of the pool a curious appearance

LITERATURE

Miall *Aquatic Insects*

Chapter X

TO CAPTURE, PRESERVE ALIVE, AND EXPERIMENTALLY FEED MOSQUITOES

To Capture Anopheles

Necessary Apparatus.—One or two small collecting tubes (Fig. 27), a clean and perfectly dry bottle (whiskey bottle), some cotton wool.

To Detect Anopheles

Choose a suitable native village, *e.g.* in Africa any bush village, or in India any village near a nullah or other source of *Anopheles* larvae.

Determine whether *Anopheles* exist in any of the following situations:—

1. In the dark corners of sheds, cow houses, or other out-houses.
2. Under the eaves (in darkish parts) of the huts.
3. In the huts themselves hanging to straws, stalactites of soot, etc., etc.
4. Any other likely situations, *e.g.* collections of dry grass, in the undergrowth in the bush (capture in this situation is difficult).

Procedure.—If on inspection none of the insects can be detected by careful scrutiny (the

most concentrated attention is, as a rule, needed), the thatch should be carefully disturbed with the hand or a short stick

Observe carefully any insects which fly out and note where they settle Choose especially portions of the thatch which are not too dark to prevent one seeing clearly, but are not too much exposed to light

Train if possible, one or more intelligent natives to detect the insects and to collect them, as shortly described It is a good thing if even only a very few *Anopheles* have been found by a personal inspection, to offer a small reward to any persons in the village who will undertake to collect them One or two tubes should be left for this purpose

To Detect Culex

Examine the walls of houses out houses, and native huts I specially examine clothes hung up in native huts Many specimens of *Culex* resting in their characteristic hunchback attitude will probably be detected Especially on dark clothing old blankets, inside leather boots or boxes

Mosquitoes seem especially fond of the smell (?) of leather

To Detect Taeniorhynchus

These are best caught by sitting with a light near a marsh or grassy land A wall or tent or cloth hung up should be at hand and kept slightly illuminated with a lamp They may be captured as they settle upon the sheet or upon oneself

To Capture Mosquitoes

1 Place a collecting tube *very slowly* over the mosquito

2 Insert a finger underneath and so rapidly block the tube or a piece of cardboard or wool may be carefully slipped underneath

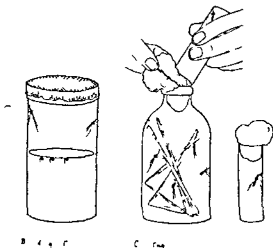


Fig. Method of Collecting and Breeding out Mosquitoes

3 Place a plug of cotton wool in the mouth of the tube

4 Transfer to the large bottle by placing the tube over the mouth of the bottle and withdrawing carefully the cotton wool Keep the bottle closed with a plug of cotton wool

5 Capture as many specimens as required and transfer them as caught to the bottle

A wide mouth bottle over which a piece of stout paper has been tied, with a small trap door cut slightly larger than the tube, may be used. This has the advantage that mosquitoes do not so easily fly out into the tube during the act of transference. It has the disadvantage that the paper tears and the mosquitoes are more likely to escape through accidental circumstances.

To Breed Out Mosquitoes

(Fig. 7)

Collect a number of full grown larvae and nymphae of both *Anopheles* and *Culex*.

1. Separate the nymphae from the larvae and place them in a jar or wide mouthed bottle half full of water, leaving room for the insects when hatched. Cover the jar with a piece of *thick cardboard or a lid, the central portion of* which is replaced by mosquito netting.

2. Place the larvae where they will receive plenty of light but will not be subject to great heat.

3. Remove the nymphae as they are seen at the end of each day.

To Keep Mosquitoes Alive

The length of time mosquitoes remain alive in captivity depends almost entirely upon the suitability of the conditions under which they are kept.

Except for special *purposes mosquitoes* (especially *Anopheles*) should *not* be kept in open spaces, *i.e.* frames covered with mosquito netting.

Procure several chutney jars with hollow

glass stoppers These can be obtained generally from the native bazaar in India for a few annas (Fig. 28) This form of jar is very convenient but any other jar will serve

Cut a piece of thick cardboard so that it will when forced down into the jar remain supported on the shoulders of the jar

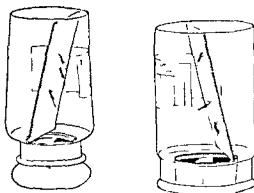


Fig. 28 Method of keeping Mosquitoes alive

Fill the stopper nearly to the brim with water Cut a thin slice of cork and place it on the surface of the water Upon the cork place a piece of clean white paper The paper should not quite occupy the whole of the space in the mouth of the stopper

1 Invert the chutney jar (prepared as above) over the top of a jar in which some mosquitoes have hatched Remove the cardboard and gently tap the glass The mosquitoes will fly upwards into the chutney jar Place the chutney jar containing mosquitoes upon its stopper prepared as above

Place the whole, after labelling, *in a dark cupboard* or other convenient place (incubator)

At the end of the first day or so, the males will be found dead upon the piece of paper, and can be removed On the second night after hatching most of the insects will feed and the jar is ready for use

2 Place the inverted (chutney) jar, prepared with cardboard as above over a bottle in which *Anopheles* caught in a village or elsewhere have been placed Remove the cotton plug and shake the bottle gently to drive the insects out Replace jar upon the prepared stopper Place in a dark spot Next morning remove the stopper and remove any *dead mosquitoes* and ova by *taking* out the piece of paper

On the second night after the mosquitoes have been collected the bottle is ready for feeding experiments On the third day generally the mosquitoes have no longer any blood remaining in the mid gut and are ready for dissection

The glands of any mosquitoes that may die before this may of course be dissected if desired on the chance of finding sporozoits

In the use of village caught *Anopheles* it must be borne in mind that any subject upon which they are fed is liable to a fresh infection In the case of natives (who sleep without hesitation in any village) the employment of village caught mosquitoes cannot however be very prejudicial

The advantages of the above way of keeping mosquitoes are —

1 The mosquitoes will keep alive longer than in any other way known to us

2 The immense convenience in feeding

3 Any mosquitoes that may have died in the night can be recovered and are not dried up

4 It is an extremely convenient way of obtaining and examining the ova

5 Mosquitoes which have become feeble are given the best possible chance of living and will be found resting all day on the piece of paper

If boxes and net covered frames be used an enormous mortality usually results The dead bodies dry up and get lost in the folds of netting or unless special precautions are taken are eaten up by ants

If chutney jars with hollow stoppers cannot be procured —

Procure any form of wide mouthed jar or bottle such as a prune jar preserved fruit bottle etc Insert a piece of stout cardboard as before

1 Prepare the metal top of a screw top bottle or some other suitable small receptacle with water cork and paper as above Place upon a square piece of very stout cardboard or wood Invert the jar over this (Fig 28)

2 Prepare a saucer by adding a few teaspoonfuls of water and placing on this cork and paper Invert the jar over the saucer This is rather more convenient than the last mentioned method as mosquitoes are less liable to escape in the process of lifting the bottle

To Feed Mosquitoes

Select a bottle in which the mosquitoes (twenty to thirty or at least a dozen in each bottle) are ready for feeding i.e. the second evening after hatching or collecting Lift the bottle from the

stopper first disturbing any mosquitoes which may be resting on the stopper, and place it mouth downwards on the table

Slip underneath the mouth of the bottle a small piece of mosquito netting of rather a fine mesh Tie this around the neck of the bottle with twine The bottle is then ready for feeding

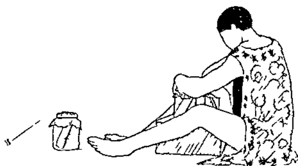


Fig 8x Method of F ding Mosquit es

At or shortly after dark t ke as many bottles as may be desired to the ward or dormi tory Slightly damp the forearm (or the calf) of the patient and turning the bottle right side up let the patient s arm re t upon the mouth of the bottle (Fig 8x)

In from a few minutes to half an hour or more the bottle will be noticed to have splashes of blood upon the bottom and sides (in the case of *Anopheles* only) If possible wait till all the *Anopheles* have fed

Remove the bottle invert upon a table untie the twine and remove the netting Replace the bottle upon the prepared stopper

Repeat the process every night allowing the mosquitoes to feed by preference on the same case throughout. Where it is uncertain which of several cases may or may not have mature sexual forms in the blood a bottle may be fed on alternate nights on the different cases.

Add clean water and a fresh piece of paper each time the bottle is used.

To Prepare Fed Mosquitoes for Dissection

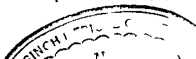
After having fed the mosquitoes in a bottle for a certain number of days place it apart from others and allow it to remain undisturbed (merely changing the water etc.) for several days.

Ascertain each day whether the mosquitoes have completely got rid of the blood in the mid gut. When they are quite free from any dark colouration of the ventral aspect of the abdomen they are ready for dissection.

N.B.—If chloroform and especially if tobacco smoke is used to kill the mosquito it is essential to well wash the jar before again keeping mosquitoes in them.

To Feed Mosquitoes on Birds, etc.

1. Prepare a framework of wood and book binders cardboard. Cover two sides with cardboard. Cover one end with netting drawn tight and to the other attach a sleeve of netting. Catch or breed out a number of *Culex* (e.g. *Culex fatigans*) and place in the frame. Keep the frame in a dark place and place a saucer of water in it.



Before placing the bird in the cage a small bag of netting should be tied around its head as it then remains perfectly quiet and further, the legs may be fastened. Small birds, such as sparrows, should be carefully treated as otherwise they are very liable to succumb. Pigeons should be treated in the same way if necessary.

2. Mosquitoes may be fed singly on pigeons and other large birds by placing the end of the test tube in which the mosquito is confined against an area of skin denuded of feathers.

Feeding Experiments on Birds

1. Feed a number of *Culex c*, *C. fatigans* on sparrows (in which have been detected proteosoma in the blood) by placing these for a time in the mosquito cage.

After feeding one or two days, place those mosquitoes which obviously have fed and are gorged with blood in a prepared chutney jar, and keep until ready for dissection.

Note (i) the zygotes of proteosoma which generally occur in large numbers in the stomach wall and in which very coarse and dark pigment is seen.

(ii) Feed some *Anopheles* on proteosoma sparrows and note that no zygotes are formed.

(iii) Feed some *Taeniorhynchus* on proteosoma sparrows and note the negative result.

(iv) Feed some *Culex* upon pigeons containing halteridium and note negative result.

Sparrows containing halteridium so frequently (in India) contain proteosoma that even if the latter is not observed under the microscope, it is difficult to be sure of their absence.

Chapter VI

DISSECTION AND EXAMINATION OF MOSQUITOES FOR THE MALARIAL PARASITE

Dissection of Mosquitoes

Necessary Apparatus

1. Slides and coverglasses.
2. Two needles, preferably the straight surgical needles described for making blood films, as they have a cutting edge.
3. Some salt solution, 0·5 grammes per cent.
4. It is convenient to have a board twelve by three inches covered half with white and half with black paper.

Some mosquitoes are caught by slipping over the top of the jar used for feeding another empty jar of the same size, and they may be kept alive in a dark cupboard for two or three days until their stomachs are quite free from blood (seen by the complete disappearance of black from the ventral portion of the abdomen).

A few specimens are killed by chloroform or tobacco smoke.

1. Observe (if a gravid female) two whitish areas on either side of the hinder portion of the abdomen (ripening ovaries). If the blood in the stomach be not digested a dark mass will be seen

in front of these, and possibly the extreme anterior portion of the abdomen will appear transparent (air containing oesophageal diverticulum) (Fig 29)

To Dissect Out the Mid Gut (Stomach)

1 Pull off with forceps the legs and wings (and remove most of the scales by a few strokes of a small camel hair brush)

2 Place a drop of salt solution on a slide and place the slide on a light background



Fig 29

Turn the mosquito upon its back and with a needle held in the left hand transfix the thorax

Carry the mosquito transfixed on the needle to the slide and lower the tip of the abdomen into the drop of salt solution

Keeping the transfixing needle in position make with the other needle, a nick upon either side between the sixth and seventh abdominal segments which point corresponds to the division between the mid and hind guts After thus loosening the last few segments place the point of the needle upon them, slowly dragging them away from the rest of the abdomen

3 After separating the segments a very short distance, remove the preparation to a dark background Again draw apart and note the white viscera stretching between them Make

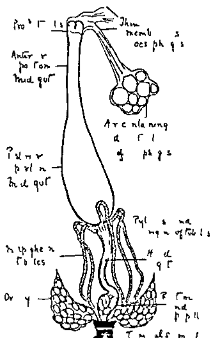


Fig. 30 Dissection of the Viscera of a Mosquito

steady traction until a central rather transparent body is alone left between the two portions of abdomen

Cut across the anterior attachments of the mid gut

4 Draw the body of the mosquito away from the separated segments the mid gut and sundry other viscera will be left attached to the latter floating in the salt solution

Observe that when the tension is relieved, the structure last to leave the abdomen of the mosquito assumes a saccular appearance This is the mid gut

THE VISCERA

(Fig 30)

1 Unless the mosquito is newly hatched note two opaque white oval bodies (the ovaries) attached to the separated segments If the ovaries are near maturity, masses of white ova are seen

2 *The Mid gut* —This extends from the level of the first pair of legs to the posterior border of the sixth abdominal segment

(i) An anterior narrow portion resembling an oesophagus

(ii) A posterior dilated portion at the level of the sixth (and fifth) abdominal segments in which, if the last meal of blood is not quite digested a black mass will be seen If any blood remains in this portion *i e* the stomach discard the specimen for one kept longer without food

(iii) At the commencement of the mid gut a ring like thickened portion (the proventriculus) It acts as a valve between the oesophagus and mid gut (Fig 30)

3 Passing between the mid gut and the separated segments note five brilliantly white threads—the malpighian tubules (Fig 30)

4. Between the malpighian tubules the transparent intestine which may exhibit active peristalsis (Fig. 30).

5. Attached to the proventriculus an exceedingly delicate membrane the dilated oesophagus and three diverticula of the same which usually contain air bubbles. They contain blood after a full meal and according to NUTTALL and SHIPLEY, these diverticula function as food *reservoirs*.

The ventral diverticulum extends as far back as the fifth abdominal segment.

TO PREPARE THE MID GUT FOR EXAMINATION.

1. Cut (by pressing with the needle) across the intestine and malpighian tubes just below the termination of the saccular mid gut. This will separate the mid gut from the rest of the viscera.

Remove everything from the slide but the mid gut. Remove excess of fluid and see that no ova or extraneous matters are left upon the slide. Add a small drop of clean salt solution and place a thin coverglass upon the preparation. The mid gut will flatten out considerably. Remove with filter paper applied to the edge of the coverglass any excess of fluid. Examine under one third inch objective and afterwards under one twelfth.

If the mid gut has been removed *in toto* and the preparation not too much compressed the following appearances are seen:—

1. The narrow anterior portion of the mid gut with the calyx like proventriculus at its free end.

2 If a portion of the extremely thin membrane of the true oesophagus or its diverticula be included in the preparation it will probably be seen to exhibit peculiar markings due probably to muscular fibres in the membrane, but resembling rather closely sporozoits It is essential that this structure should be recognized when seen and that the resemblance of its markings to sporozoits should not lead the beginner astray (Fig 32)

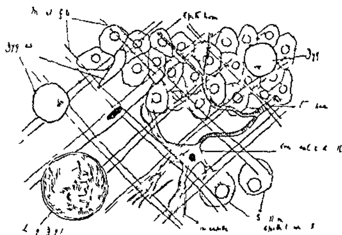


Fig 31 Microscopic appearance of Mid gut showing Cell Structure and Zygote

3 The expanded posterior portion of the mid gut This body forms the main mass of the preparation, and is all important in relation to malarial studies

The following appearances are seen in a good preparation —

1 Well defined tubes with spiral lining (air tubes or tracheae) Note that these branch and

ramify upon the surface of the mid gut and malpighian tubes (Fig 31)

2 Large muscular fibres together with elastic fibres forming a check pattern Note that they are circular and longitudinal (external) Note that at the edge of the viscus they are seen in optical section (Fig 31)

3 Large cells with large nuclei and granular protoplasm (epithelium of mid gut) Note some *in situ* forming a single layer of polygonal epithelium and others detached and in process of being carried along by fluid streaming from interior of mid gut Note that in some places these cells are undergoing vacuolization with dancing of the protoplasm granules (Fig 31)

4 Note any contents of the stomach—

(i) Remains of blood

(ii) Crystals of various kinds

(iii) Gregarines flagellates bacteria etc

5 Note that in focussing downward one passes through a double thickness of wall Note that the air tubes are focussed on the upper and lower surfaces of the preparation and the epithelium and crystals in the middle

6 Trace several of the finer air tubes to their apparent termination and note that when they lose their spiral lining they are continued as very fine transparent tubules (air capillaries) Note that at the point of breaking up one can generally make out large stellate cells (tracheal cells) (Fig 31)

7 Observe in some preparations large oval cells of brownish colour lying upon the outer surface of the stomach Note that they are rather

opaque and contain a certain amount of diffuse yellowish pigment They are so called pericardial cells (see Fig 32)

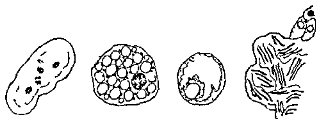


Fig 32 (Left to right) Pericardial Cell Fat Body Cell Swollen Epithelium Cell Thin Membrane showing Sporozoit like appearance

8 Observe in most preparations one or more large clear cells with a small nucleus and filled with oil globules (cells of the fat body) (Fig 32) These lie upon the stomach and in common with the last named cells are accidental in this situation

The Examination of the Mid Gut for the Zygote or Oocyst Stage of the Malarial Parasite

(The examination of the stomach blood for flagellating and the motile or vermicule forms is deferred to a later Chapter)

Obtain a number of *Anopheles* (not *M Rossii*) from some native quarter (see p 92), or better, those specially fed Keep these alive for two or three days until no blood remains in the mid gut (for methods of keeping alive, see p 95)

Prepare the mid gut as described above. A considerable number may prove negative but a variable percentage will be positive. Examine with one twelfth inch.

Carefully note the presence of small collections of pigment of the nature of *malarial pigment*. By careful focussing the younger forms may be seen as clear oval or round bodies 6–7 μ in which the distinct and clearly defined pigment occurs. The more advanced forms can scarcely be missed. It is necessary to bear in mind the normal structures and the fact that until the parasite reaches a considerable size and has a very sharply defined cyst wall, pigment of the characters belonging to the species of parasite concerned is present.



Fig. 33. *(Left to Right) Zygotes of Malignant Tertian, Simple Tertian,* [illegible]

1. Zygotes of crescent tertian shew when young a clump of pigment resembling black pepper grains (Fig. 33).

2. Zygotes of simple tertian shew yellowish or golden pigment in wisps (Fig. 33).

3. Zygotes of quartan shew rather coarse pigment in a clump (Fig. 33).

The older zygotes (40–60 μ) are indistinguishable as regards the species of parasite concerned. They may shew:

1. A very clear and distinct cyst wall (adventitious).

2 The formation of sporoblasts

3 In still more developed forms, the sporoblasts are seen to be surrounded by a radiating arrangement of young sporozoits or blasts (Fig 9 and 31)

4 Fully developed forms are large cysts packed with many hundreds of fine sickle shaped bodies, and, if they are ruptured, these latter escape into the surrounding fluid and are readily distinguished as sporozoits (Fig 37)

To Make Permanent Preparations of Zygotes

Method 1 —In case of a specimen shewing zygotes place a large drop of two per cent formalin on one side of the coverglass, and draw this through by filter paper placed on the other side Repeat several times Remove excess of the formalin with filter paper Ring edges of cover glass with black varnish or Canada balsam

The zygotes will retain their appearance as seen in the fresh specimen

Method 2 —Run formalin through as in Method 1 When an excess of fluid is present, slide off the coverglass The flattened mid gut will probably remain attached to the coverglass

Wash in water and stain lightly with methylene blue Wash in water, and allow to dry Warm gently to ensure complete dryness, and place the coverglass mid gut downwards upon a drop of balsam upon a slide

The muscular fibres and other structures of the mid gut will be well exhibited The zygotes will be stained rather a dark blue If not too

darkly stained the pigment of the zygotes will have the appearance it had in the fresh specimen

3 Mount directly in glycerine

4 For more minute histological examination imbed the stomachs in paraffin or the whole mosquito (*vide* p 123)

To Dissect Out the Salivary Glands

This is quite a simple proceeding if it be remembered where they lie They are intra thoracic structures and they commence at the hinder portion of the neck and end opposite the first pair of legs They lie ventrally in fact roughly speaking they lie just above the origin of the first pair of legs (Fig 34)



Fig 34 Showing Salivary Glands

The simplest and most rapid method and the one that hardly ever fails is the following

1 Place the mosquito in a drop of salt solution on its right side with the head pointing towards you as you dissect

2 Place the needle of the left hand on the thorax to steady it, and place the needle of the right hand on the back of the head and make steady *gentle traction*

3 If done carefully, it will be seen that the head has pulled out a little mass of white tissue from the thorax (the dissection is best done on a dull black surface)

4 Examine the piece of tissue under a half inch lens (The diaphragm should be as nearly closed as possible) The glands will be seen hanging on to the neck as finger like, transparent, *glistening* bodies Muscle has a greyish look and even the fat body is not so refractile as the glands

5 Now place one needle on the head and with the other make a transverse cut between the head and the attached portion of the glands

6 Examine again the now separated glands Generally all six are with certainty got in this way

7 If the glands are not found on the neck proceed with the dissection by Method 2

8 When dissected out in this way they are generally quite free from surrounding tissues, but if found necessary they can be teased out further and placed in fresh drops of salt solution

9 At all stages of the dissection make sure *that the glands are really present* and that they have not floated to the side or stuck to the needles

10 *By this certain and rapid method* as many as one hundred glands may be dissected out put under a coverglass and examined microscopically in a day's work

Method 2.—Consists in isolating, by a series of cuts, the anterior ventral portion of the thorax in which the glands lie.

1. Make a cut obliquely in an antero-posterior direction so as to sever the main mass of thoracic muscle.
2. Make a cut at right angles to this, passing just behind the attachment of the first pair of legs.
3. Cut through the neck.
4. The glands lie in the portion thus isolated. Considerable teasing out is still required to isolate them from the surrounding tissues. Examine each portion of tissue separated out and *remove to a fresh clean drop of salt solution.*

Remember that in examining under the microscope the apparent right hand is really the left and *vice versa.*



Fig. 35.—The Salivary Glands

This method, which is longer than Method 1, requires more dissection and teasing out in order to isolate the glands cleanly, and, as we have said, may still be followed even if No. 1 has failed; but our experience has been that Method 1 is learned at once without any difficulty.

Ascertain that the glands of either side consist of three acini the ducts of which join almost immediately after leaving the acinus to form a single long duct

1 Observe that of the three glands of each side (Figs 35 and 38) —

(i) Two are highly refractile, and the cells in these are very distinct and clearly defined (lateral glands)

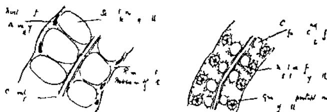


Fig 36 Microscopic Structure of Salivary Acinus and that of a Newly Hatched Mosquito (right)

(ii) One is much less refractile and the component cells are much less easily defined (central gland)

2 Observe that each acinus has a duct running through its whole length, and that the *secretory cells form a single row around this*

3 Observe that each secretory cell has a large mass of clear secretion within it, forming the chief bulk of the cell and that the nucleus is flattened and pushed to the periphery (Fig 36) Pressure tends to force the secretion out of the cell in viscid looking droplets The secretion of the *lateral glands is far more refractile than that of* the central (Fig 38)

4 Ascertain that the duct formed by the junction of the three intra acinar ducts joins,

eventually the similar duct from the other side to form a common salivary duct which passes into the salivary receptacle. The duct is thick walled and is lined with a spiral thread resembling a tracheal tube.

EXAMINATION OF THE SPOROZOIT FORM OF THE MALARIAL PARASITE

Obtain a number of *Anopheles* (not *M. Rossii*) from a native quarter (five per cent to twenty per cent or more have sporozoits in the glands) or *Anopheles* fed for twelve days or more at a temperature of 80 F. Prepare specimens of the glands as described above. Having placed one or more lobes under a low power, press with the point of a needle on the cover-glass, so that the gland is ruptured and the secretion poured out as droplets into the surrounding fluid.



Fig. 3.—Sporozoits in the Salivary Gland (Low Magnification)

Examine with one sixth inch. If sporozoits are present they are generally very numerous and large numbers of fine very distinct curved rods, will be easily seen with this power lying throughout the fluid around the gland and packed in large numbers in the substance of the gland. Finally examine with one twelfth inch (Fig. 37).

The sporozoits have a mean length of $14\,\mu$ and vary between $10\,\mu$ and $20\,\mu$ and are $1{\cdot}2\,\mu$ in width.

EXAMINATION OF MOTION OF SPOROZOITS

Dissect out the glands and when isolated cleanly transfer to a drop of human serum, previously got ready by allowing blood to clot in a small tube. Three kinds of motion may be observed:—

1. Formation of curves.
2. Formation of ring formed contractions.
3. Locomotion. Forward motion.

Penetration of Red Cell by Sporozoits.—This has not been seen in case of sporozoits of the salivary glands but has been observed twice by SCHAUDINN in the case of sporozoits from a ruptured cyst in the stomach.

TO PREPARE PERMANENT PREPARATIONS OF SPOROZOITS

Pressing firmly upon the coverglass draw it along the slide so that a film is made on coverglass and slide.

Dry by rapidly waving the slide and the coverglass in the air. Fix both in alcohol, and

stain with Romanowsky. Wash, dry, and examine without coverglass with an oil immersion.

The sporozoits appear as fusiform bodies with a central red mass of chromatin. They are about 14 μ in length, with one end often more pointed than the other.

Wash off the oil with xylol, dry, and label.

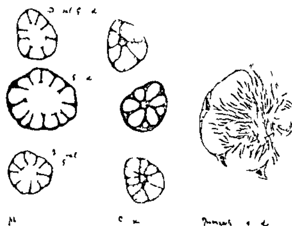


Fig. 38.—Sections of Salivary Glands, showing differences between the Central and Lateral Glands and Lobes in the ... of Anophelines and Culicines, ... Sporozoits in the Gland of Anopheles.

The Reproductive System

To Examine the Spermatheca (Fig. 39).—By pressing with the edge of the triangular needle cut off the extreme tip of the abdomen, the last or eighth segment only. Place this in a very small drop of salt solution and tease the fragment

carefully apart A small black granule (sometimes two) will be seen Isolate this as much as possible from other tissue and cover with a cover glass

Observe under a low power a brown chitinous ball Press firmly on the coverglass so as to rupture it *Examine under one twelfth inch*

Observe the masses of fine hair like actively motile bodies if (as is probably the case) the mosquito has been fertilized Isolate some of these they possess the characters of spermatozoa (Fig 39)

Examine the spermatheca of a mosquito newly hatched it does not contain spermatozoa

To Examine the Ovaries

Examine the ovaries of a number of mosquitoes caught in the room, etc

Observe that when the ovaries nearly reach maturity they are readily detected as white areas on either side of the posterior part of the abdomen and that when fully developed they occupy the whole of the lateral and dorsal portions of this

Drag off the last few segments of the abdomen in a drop of salt solution and allow the ovaries to float out in this Observe that they are *pyriform bodies the apex being above* (Fig 39)

Ascertain that each ovary consists of a large number of follicular tubes commencing as fine *threads and ending in the oviduct* Observe especially the follicular tube forming the apex of the ovary as here this is most readily made out

Ascertain that each follicular tube contains several egg follicles the lowest of which is the most advanced in development

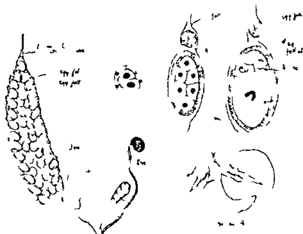


Fig 33 Spermatheca with Spermatozoa Structure of Ovaries

Note that these latter show various stages of development in the different mosquitoes Note that each consists of an outer layer of small cubical cells (follicular epithelium) and an inner mass composed of from four to eight large cells Note the first appearance of the yelk as small oil globules in the lowest cell (the ovum) and how this increases until it nearly fills the follicle by encroaching on the other cells (nurse cells)

Observe in the mature ovum that the outer covering is formed of the original layer of outer cells (follicular epithelium)

The following organisms may be found in mosquitoes apart from the various stages of the malarial parasite —

1 Encysted trematodes mostly found in the tissues near the neck

2 Nematodes in the thorax or abdominal cavity

3 *Sporozoa* (*a*) Sausage shaped bodies in masses, sometimes in close connexion with the salivary glands (Fig 40)

(*b*) Octosporozoa, consisting of eight small sausage shaped bodies in small cysts enormous numbers of which replace the yolk of the ovum (Fig 40)

(*c*) Flagellate bodies in the rectum and hind gut frequently in enormous numbers, and shewing when stained large numbers of developmental forms



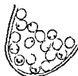






Fig 40 Protozoa other than the Malarial Parasite found in Anopheles

(*d*) Gregarines. In the adult these have become encysted in the malpighian tubes. In the larvae the worm-like gregarine will be found actively motile in the malpighian tubules.

4. The developing embryos of filariae. These are seen as sausage-shaped bodies with a terminal spike in the dissection of the salivary glands. In sections of mosquitoes they are seen in the muscles, especially in those of the thorax.

5. The developed embryos of filariae in the labium or about the base of the neck.

6. In the oesophageal diverticula masses of micro-organisms and sporozoa (?) will be found.

To Cut Sections of Mosquitoes.

1. Kill some *Anopheles* by tobacco smoke or chloroform, or allow them to fall directly into absolute alcohol by pouring a few drops into the tube containing them. Avoid if possible mosquitoes containing blood, as the blood becomes very hard.

2. Allow to remain in alcohol fifteen minutes to harden somewhat.

3. Remove one by one. Cut off with a fine scissors the legs and wings. Make a minute incision into both the thorax and abdomen, e.g., holding the mosquito carefully between the finger and thumb slice off with a sharp razor a portion of the dorsum of the thorax and a minute portion of one side of the abdomen. This allows more perfect penetration of fluid.

4. Replace in absolute alcohol (some authors recommend bathing in alcohol as the air in the

[illegible]

The following organisms may be found in mosquitoes apart from the various stages of the malarial parasite —

1 Encysted trematodes, mostly found in the tissues near the neck

2 Nematodes in the thorax or abdominal cavity

3 Sporozoa (*a*) Sausage shaped bodies in masses sometimes in close connexion with the salivary glands (Fig 40)

(*b*) Octosporozoa, consisting of eight small sausage shaped bodies in small cysts enormous numbers of which replace the cell of the ovum (Fig 40)

(*c*) Flagellate bodies in the rectum and hind gut frequently in enormous numbers, and shewing, when stained large numbers of developmental forms



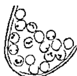






Fig 40 *Protozoa other than the Malarial Parasite found in Anopheles*

(*d*) Gregarines. In the adult these have become encysted in the malpighian tubes. In the larvae the worm-like gregarine will be found actively motile in the malpighian tubules.

4. The developing embryos of filariae. These are seen as sausage-shaped bodies with a terminal spike in the dissection of the salivary glands. In sections of mosquitoes they are seen in the muscles, especially in those of the thorax.

5. The developed embryos of filariae in the labium or about the base of the neck.

6. In the oesophageal diverticula masses of micro-organisms and p rozoa ( ) will be found.

To Cut Sections of Mosquitoes

1. Kill some *Anopheles* by tobacco smoke or chloroform, or allow them to fall directly into absolute alcohol *by pouring a few drops into the* tube containing them. Avoid, if possible, mosquitoes containing blood, as the blood becomes very hard.

2. Allow to remain in alcohol fifteen minutes to harden somewhat.

3. Remove one by one. Cut off with a fine scissors the legs and wings. Make a minute incision into both the thorax and abdomen, e.g., holding the mosquito carefully between the finger and thumb, slice off with a sharp razor a portion of the dorsum of the thorax and a minute portion of one side of the abdomen. This allows more perfect penetration of fluids.

4. Replace in absolute alcohol (some authors recommend boiling in alcohol, as the air in the

[illegible]

tracheae is expelled, and the alcohol then pene trates completely) allow to remain one to two hours This which is to ensure complete dehydra tion is the most important of operations, and upon it depends the success of the embedding Make two or three changes, using alcohol to which $CuSo_4$ has been added

5 Remove from alcohol and place for ten minutes or so in xylol When the thorax becomes transparent, the mosquito should be removed, as too long a time in xylol produces much hardening

6 Place for ten minutes in paraffin (*vide* p 46) kept melted in a watch glass on the heated metal slab

7 Smear a clean watch glass with a little glycerine and fill with paraffin heated somewhat over melting point transfer the specimen to this with a warm forceps to avoid cooling the paraffin

8 Arrange the specimen as required As soon as the surface of the paraffin has set, plunge the whole mass into water, as paraffin rapidly *cooled is more homogenous*

If the watch glass has been smeared with glycerine it will be easy to remove the block

9 Cut out the specimen arrange as desired for cutting transversely, vertically or horizontally Take care that the top and bottom edges of the block are parallel Smear these with a little melted soft paraffin (made by melting a little hard paraffin with vaseline) This gives an unbroken ribbon of sections

To Mount Sections of Mosquitoes

Unless the sections are thick it is necessary to flatten them

Heat some water in a dish a little below the melting point of the paraffin. Test the right temperature by dropping a section on the surface. If it melts the water is too hot. If it does not flatten out the water is not hot enough.

Drop upon the surface a ribbon of five or six sections; it will become perfectly flat if the temperature is right.

1. Smear some slides with solution of pyroxylon in oil of cloves (Appendix), taking care to avoid an excess. Lower the slide rapidly into the water and with the aid of a needle draw it out again with the sections lying flat upon it.

2. Press lightly but firmly between smooth blotting paper. Protect from dust and allow to dry for twenty four hours or dry more rapidly over the flame, but avoid melting the paraffin.

When dry, hold the slide a few seconds over the flame; this drives off the oil of cloves and melts the paraffin.

Pour over the slide first xylol, then alcohol, and finally place in water.

Mosquito tissues are so delicate that in mounting it is difficult to avoid the separation of portions or the whole of the section. This is especially so in the case of the chitin, which frequently breaks away.

OBREGIA'S method is recommended as giving excellent results.

1. A slide is smeared with a thin film of a mixture consisting of two parts of commercial liquid glucose and one part of a solution of dextrin (dextrin 16 oz., water 17½ oz., thymol 15 grains).

trachere is expelled, and the alcohol then penetrates completely), allow to remain one to two hours This, which is to ensure complete dehydration, is the most important of operations, and upon it depends the success of the embedding Make two or three changes, using alcohol to which $CuSo_4$ has been added

5 Remove from alcohol and place for ten minutes or so in xylol When the thorax becomes transparent the mosquito should be removed, as too long a time in xylol produces much hardening

6 Place for ten minutes in paraffin (*vide* p 46) kept melted in a watch glass on the heated metal slab

7 Smear a clean watch glass with a little glycerine, and fill with paraffin heated somewhat over melting point, transfer the specimen to this with a warm forceps to avoid cooling the paraffin

8 Arrange the specimen as required As soon as the surface of the paraffin has set, plunge the whole mass into water, as paraffin rapidly cooled is more homogenous

If the watch glass has been smeared with glycerine it will be easy to remove the block

9 Cut out the specimen arrange as desired for cutting transversely, vertically or horizontally Take care that the top and bottom edges of the block are parallel Smear these with a little melted soft paraffin (made by melting a little hard paraffin with vaseline) This gives an unbroken ribbon of sections

To Mount Sections of Mosquitoes

Unless the sections are thick it is necessary to flatten them

Heat some water in a dish a little below the melting point of the paraffin. Test the right temperature by dropping a section on the surface. If it melts the water is too hot. If it does not flatten out the water is not hot enough.

Drop upon the surface a ribbon of five or six sections; it will become perfectly flat if the temperature is right.

1. Smear some slides with solution of pyroxylon in oil of cloves (Appendix), taking care to avoid an excess. Lower the slide rapidly into the water and with the aid of a needle draw it out again with the sections lying flat upon it.

2. Press lightly but firmly between smooth blotting paper. Protect from dust and allow to dry for twenty four hours or dry more rapidly over the flame but avoid melting the paraffin.

When dry hold the slide a few seconds over the flame; this drives off the oil of cloves and melts the paraffin.

Pour over the slide first xylol then alcohol and finally place in water.

Mosquito tissues are so delicate that in mounting it is difficult to avoid the separation of portions or the whole of the section. This is especially so in the case of the chitin which frequently breaks away.

OBRECCIA's method is recommended as giving excellent results:—

1. A slide is smeared with a thin film of a mixture consisting of two parts of commercial liquid glucose and one part of a solution of dextrin (dextrin 16 oz, water 17½ oz, thymol 15 grains).

Chapter VII

INTERNAL ANATOMY OF MOSQUITOES

The Alimentary Canal

The alimentary canal is specialized on account of the blood sucking habits of the mosquito It differs from many insects in not possessing any caecal diverticula of the mid gut It also differs in the possession of five malpighian tubules these being in insects usually even in number (Fig 30)

The parts of the alimentary canals are as follows —

The mouth The pharynx with pumping organ The oesophagus The oesophageal diverticula	The fore gut
The homologue of the proventriculus The stomach (so called) The pylorus	*The mid* gut
The pyloric dilatation The small intestine The colon The rectum with rectal papillae	The hind *gut*

The mouth, pharynx and oesophagus are ectodermal in origin and both the mouth and pharynx are *lined with chitin* The hind gut is also ectodermal in origin it does not possess, however, any portion lined with chitin The

mid gut is the true digestive portion of the tract

The Pharynx —The pharynx which is lined throughout its extent with chitin passes upwards and backwards through the ganglionic ring formed by the supra and infra oesophageal ganglia and their commissures At first it is narrow but posteriorly becomes a large chamber (the pumping organ) (Fig 43A)

The pumping organ occupies with its muscles a large portion of the head behind the level of the cerebral ganglia In the state of rest its lumen is triradiate in transverse section The walls are formed of three large and thick chitinous plates one placed on either side and one superiorly Into each of these plates powerful muscles are inserted Those of the superior plate consist of two muscular masses taking their origin from the occiput Those of the lateral plates consist on each side of a single large muscular mass arising from the lateral portions of the head The plates are connected by thin non chitinous membrane and their edges are rolled so that they form a spring capable of returning to their original position so soon as the separating force of the muscles ceases

Posteriorly where the pharynx becomes very narrow a sharp bend occurs and a valvular action is produced The whole forms a very powerful suctorial apparatus

The Oesophagus —Immediately beyond the pumping organ the chitinous layer ceases and the rest of the fore gut is formed of excessively thin membrane At the junction of the two portions a sharp bend occurs and the floor projects so as to form a valvular flap

The thin walled oesophagus is a large dilated sac whose walls are supported by surrounding structures Into the posterior wall of the dilated and thin walled oesophagus projects the papilla like anterior portion of the mid gut

The Diverticula of the Oesophagus—From the oesophagus two or three diverticula similar in nature to the oesophagus extend backwards Of these one is of great size and usually contains air This most usually extends into the abdomen and is a prominent object in dissections and sections In the newly hatched mosquito it is small but rapidly becomes large enough to extend into the abdomen (Fig 30)

The Homologue of the Proventriculus—There is no true proventriculus as in many insects There is however an interesting fold of the fore gut into the mid gut which represents this organ The anterior portion of the mid gut has been noted as projecting into the dilated oesophageal pouch This portion consists of both ectodermal and endodermal portions and represents the proventriculus in other insects The muscular bundles are here increased and the whole forms a valvular muscular organ (Fig 30)

The Mechanism of Feeding—The powerful pumping action which must result from a drawing asunder of the three large chitinous plates of the pumping organ is very evident These plates, also when drawn apart must by reason of their spring like shape revert to their original positions close together without any muscular aid Posteriorly the valve like arrangement mentioned before prevents regurgitation

The Mid gut—The mid gut extends from the

proventriculus to the origin of the malpighian tubes. It consists of two portions which merge into one another—an anterior narrow portion and a large dilated posterior portion which becomes greatly distended after feeding. Unlike most insects there are no caecal appendages in the mosquito. Posteriorly there is a marked constriction with strong muscular bundles which forms a very marked pylorus (Fig. 30).

The anterior narrow portion of the mid gut lies in the thorax and does not become distended with blood. The posterior portion when fully dilated fills the greater portion of the abdomen, the viscera being pushed into the last few segments.

The Hind gut.—The hind gut is short and passes in one or two bends from the pylorus to the anus. Immediately beyond the pylorus there is a considerable dilatation which is poorly supplied with muscular fibre; into this open the five malpighian tubules. For a short distance beyond this the lumen is narrow (small intestine) but become gradually larger (colon). At the termination of the colon there is a slight constriction after which the canal dilates again to form the rectum (Fig. 30).

Into the rectum project six solid growths, the so-called rectal glands, which are however papillae. Posteriorly the rectum ends in the anus close above the gynaephoric canal.

The appendages of the alimentary canal are:—

The Salivary Glands.—The salivary glands consist of six tubular acini lying three upon either side. Those of one side lie generally one above the other in the long axis of the body, their

interior ends lying close against the prosternum where the ducts coming from each acinus unite to form a single duct. The upper and middle acini generally lie with their distal ends close to the proventriculus. The lower acinus passes towards the thoracic ganglion. Occasionally an acinus becomes bifid at a short distance from its termination. A common abnormality also is a small accessory acinus near the proximal end of an acinus. A duct can be seen traversing almost the entire length of each acinus. Shortly after leaving the acinus, the three unite to form a single duct. The duct of each side passes up into the neck and lies close to the nerve cords passing between the thoracic and the cerebral ganglia. Beneath and in contact with the lower surface of the suboesophageal ganglion the ducts of each side unite to form a common salivary duct which passes forwards and enters the chitinous first portion of the alimentary canal close to the base of the proboscis (Fig. 34).

The Malpighian Tubules.—These are five in number and open into the first portion of the hind gut immediately beyond the pylorus. Their blind ends are held in position in the neighbourhood of the rectum by tracheal branches. They pass forwards in loops above their origin, so that in transverse section as many as ten may be seen cut across.

THE MUSCULAR SYSTEM

The chief muscular masses in the mosquito are contained in the thorax. They are chiefly muscles moving the wings and legs.

Wing Muscles.—There are two large muscular

masses on either side of the thorax passing from the dorsal to the ventral body wall Between these bundles there is a space in the lower portion of which lies the alimentary canal main air tubes and other structures The upper portion of the space is occupied by a second series of large muscular bundles passing from the front to the back of the thorax Neither of these large masses of muscle are inserted directly into the wings the up and down movement of the wings being caused by alterations in the shape of the thorax consequent on the contractions of the vertical and horizontal fibres respectively

There are however a few fibres arising from the lateral portions of the thorax and inserted about the base of the wings

Leg Muscles—These occupy but little space in the thorax They rise to a large extent from the internal processes of the exoskeleton (apodemes) and are inserted into neighbouring portions of the limbs They arise also from one segment of a limb and are inserted into another

The Muscles of the Body Segments—These arise from one segment and are inserted into the next They are arranged dorsally and ventrally in lateral groups throughout the abdomen

A small muscle is also situated on each side passing vertically from the tergum to the sternum These on contracting flatten the abdomen

Muscles in Association with the Alimentary Canal—Several important muscular masses are connected with the large chitinous pumping organ A pair of muscles arises from the occipital region of the exoskeleton and is inserted into the upper plate of the organ A large muscle arises on each side and is inserted into each of the lateral plates

Loop branches passing to the trunks anterior and posterior

Branches passing inwards and supplying viscera Branches from the first second third and fourth abdominal tracheae supply mainly the mid gut those from the fourth and fifth the ovaries those *from the sixth and seventh the* genital organs

The Vascular System—As in most insects where the respiratory system ramifies throughout the whole body the vascular system is not well developed A dorsal vessel or heart and an anterior prolongation of this (aorta) are the only closed blood vessels Apart from the dorsal vessel the blood circulates in large blood spaces which lie between the lobes of the fat body and among the muscles and viscera

The dorsal vessel passes close beneath the tergal plates throughout the abdomen It is very *thin walled and is not provided with valves The* upper portion is attached to the dorsum at intervals by suspensory fibres (muscular) so that a festooned appearance is given in longitudinal section There is however no true division into compartments Laterally large cells (pericardial cells) are arranged throughout its entire extent and fibres of a muscular nature (alary muscle) pass from the body wall and end in branches in close connexion with the dorsal vessel

At the first abdominal *segment the dorsal* vessel dips down beneath the mesophragma lying as it does so in direct contact with the *cuticle* In the thorax it again arches upwards and lies between the lower portions of the antero posterior wing muscles close above the anterior portion of the mid gut

In the anterior third of the thorax it divides into two smaller portions which pass outwards and coming in contact with the salivary ducts enter the neck.

Blood spaces without definite walls occur throughout the body. The thorax especially contains large spaces among the muscles and the complex fat body which lies between and supports the organs is everywhere bathed with blood fluid.

THE NERVOUS SYSTEM.

The ganglionic system in the *Culicidae* is considerably developed. The head ganglia are large and complex. The thoracic ganglia are large and compressed so as to form a large ganglionic mass. The ganglia of this system are as follows:—

(*a*) Lying around the pharynx is a ganglionic ring composed of large supra and infra oesophageal ganglia with their commissures. From these large nerves go to the eyes, antennae and mouth parts.

(*b*) In the thorax lying below the oesophageal diverticulum and close to the sterna is a large compound ganglion showing evidence of its origin from the conjoined ganglia. Between this and the head ganglia are two long slender nerve cords which pass in the neck in close relation with the salivary ducts. From the thoracic ganglion large nerves pass to the limbs and posteriorly nerve cords connect it with the first abdominal ganglion.

(*c*) The abdominal ganglia lie with their connecting commissures close upon the abdominal

sterna The last ganglion lies just below the junction of the oviducts to form the common oviduct A large nerve passes from it among the viscera of the last few segments

The Visceral System—Small ganglia connected with the main ganglionic system occur in connexion with the viscera The most important of these are two small groups of large nerve cells lying in front of and above the thoracic ganglion *with the middle portion of* which they are connected by nerves They lie laterally beneath the oesophageal diverticulum and anterior portion of the mid gut and are not far removed from the salivary glands Another small ganglion occurs above and in front of the proventriculus (Fig 34)

The Reproductive System

The organs of the reproductive system are —

1 Ovaries
2 Oviducts and common oviduct
3 Mucus gland and duct
4 Spermathecae and ducts

The ovaries occupy a variable position dependent upon the state of their development In the newly hatched mosquito they are small bodies lying in the fourth and fifth abdominal segments close by the posterior portion of the mid gut and attached to the body wall by numerous tracheae As they enlarge they push the mid gut hind gut and malpighian tubes *towards the ventrum*, so that eventually the ovaries occupy nearly the whole of the posterior portion of the abdomen Each ovary consists of very many follicular tubes each containing egg follicles in different stages of

development In the mature ovary the lower follicles have in every tube become the large completely formed egg (Fig 39)

The oviducts are muscular tubes passing from the ovaries They join beneath the rectum to form the common oviduct which is still more abundantly supplied with muscle fibres and which eventually opens beneath the anus

The spermatheca is a chitinous sac which in the impregnated female is filled with a mass of spermatozoa Its duct is long and twisted and opens into the common oviduct near its termination

The mucus gland globular or ovoid in shape opens by a short duct into the same region

The Fat body —The adipose tissue is disposed in two ways

1 As a general lining to the body wall being nearly everywhere present directly beneath the cuticle and

2 As lobular masses lying in among the organs and muscles Thus a large pad lies over the compound thoracic ganglion and sends processes which lie in among the salivary glands and other viscera Other smaller masses lie in the head and abdomen

Histology

Methods —The examination of the fresh tissues frequently reveal structure not easily seen in fixed preparations The tissues are best dissected out in normal saline of low tonicity 0 3 or 0 4 per cent as insect juices have a lower isotonic point than those of mammals Better preparations of

a double thickness of mid gut wall as well as the fore gut There is an increase in the muscular fibres of the mid gut at this point, especially the circular fibres so that a very distinct mass is formed homologous to the *proventriculus of many* insects There is no chitinous development however, and the structure would appear to act only as a muscular sphincter (Fig 30)

The Hind gut —The nature of the epithelium and arrangement of the muscular fibres differs somewhat in different portions of the hind gut Structurally the small and large intestine are similar whilst the *dilatation beyond the pylorus* and especially the rectum differs from these

The dilatation which occurs at the origin of the malpighian tubules is thin walled and poorly supplied with muscle fibres The cells lining it are small and flattened

The intestine is lined with a single layer of large cubical cells external to these is a muscular coat The cells of the intestine have large nuclei which have a similar though more open arrangement of the chromatin than the nuclei of the mid gut The protoplasm is finely reticular and stains less deeply than the cells of the mid gut Stained with HEIDENHAIN'S haematoxylin, no granules are present as in the cells of the mid gut They have no striated border

In the rectum the cells become small and flattened There are here however bodies usually termed rectal glands These are papillae covered with a single layer of much hypertrophied cells resembling those lining the small intestine and colon

The muscular system of the hind gut is very

similar to that of the mid gut consisting of very large fusiform striated cells arranged circularly and longitudinally. The circular fibres in the small intestine lie outside the longitudinal and pass spirally around the mid gut. Towards the termination of the intestine longitudinal fibres also lie outside the circular. In the rectum and extending throughout the hind gut and mid gut in both *Anopheles* and *Culex* there are in a large proportion of specimens swarms of a flagellate organism (Fig. 40).

The Salivary Glands.—The salivary acini lie in a cleft in the fat body which latter comes in close contact with the glands. Each gland acinus consists of a single layer of large cells limited externally by a delicate sheath (basement membrane) and internally by the intra-glandular duct wall.

In *Anopheles* the intra-glandular duct becomes larger as it approaches the termination of the acinus and forms a large cavity.

In *Culex* the duct remains of the same diameter throughout the acinus and terminates abruptly near the end of the acinus without any dilatation.

In both *Culex* and *Anopheles* there are two types of gland acinus. These are recognizable both in the fresh gland and in fixed specimens. From their appearance in the latter they may be termed

(1) The granular type.

(2) The clear or colloid-like type.

The Granular Type.—The greater portion of the acinus consists of cells whose nucleus and protoplasm has been pushed to the outer portion

I

of the cell by a large mass of secretion, which occupies almost the whole of the cell In the fresh gland this secretion appears as a clear refractile substance, and can, by pressure be made to exude from the cell in refractile globules In specimens hardened in alcohol this clear secretion appears as a granular mass occupying the greater portion of the cell It stains faintly with haematein and shows *under high powers (one sixteenth* oil immersion) a coarse reticulum and isolated globules an appearance probably due to the precipitation or coagulation of the secretion by the alcohol *Considerable variations exist however,* in the appearance of this granular secretion both in the different mosquitoes and in different parts of the same gland In *Anopheles* the greater portion of the gland contains cells densely crowded with granular material Very frequently however the terminal portion contains cells in which only a few large globular masses exist (Fig 38)

The protoplasm of the cell occupies in the fully matured gland only the extreme periphery, and the nucleus which is much degenerated is pushed to the outer portion of the cell and usually lies in the angular interval left at the base of two or more contiguous cells In the granular type of gland this disappearance of the protoplasm and nucleus from view is more pronounced than in the clear type of gland

The Clear or Colloid like Type —Of the last mentioned type there are two acini upon either side of the present type there is but a single acinus upon either side which usually lies between the two acini of granular type (Fig 38)

In the fresh gland the cell outlines are not so

distinct as in the granular type, and the secretion when extended by pressure is much less refractive. In alcohol hardened specimens the acinar cells contain a large mass of clear homogenous secretion which, as in the last mentioned type, fills almost the entire cell, and pushes the protoplasm and nucleus to the periphery.

In the clear type, however, the protoplasm is always in greater amount than is the case with the granular type, and the nucleus never becomes so greatly degenerated. The clear homogeneous secretion stains readily with haematein, and may even stain quite deeply. With HEIDENHAIN'S haematoxylin it frequently becomes almost black. It resembles very much in appearance colloid substance as it is seen in the mammalian thyroid.

In *Anopheles* this substance also distends the central duct space within the acinus. In this situation an appearance is sometimes produced which resembles faintly stained sporozoites, but which is a normal condition.

The Maturation of the Glands.—In freshly hatched mosquitoes both types of acinus consist of large glandular cells arranged round the lumen. These contain a large centrally situated nucleus, and have protoplasm containing a large number of coarse granules staining with haematein. In the portion of the cell nearest the lumen a vacuole of varying size is situated. This is the commencement of the large mass of secretion which in the mature gland occupies the entire cell. In the granular type of acinus the vacuole contains granules; in the clear type it resembles the colloid-like secretion (Fig. 36).

Further Variations in the Cells of the Salivary

Acini.—In the granular type of gland the greater portion of the acinus is composed of cells of the character described above. A portion, however, usually exists which differs considerably in structure. This portion adjoins the duct, and may in *Anopheles* reach as much as one quarter of the entire gland in length. In this portion of the gland the cells are much smaller than those containing the granular secretion, so that the diameter of the acinus is much less here, and a sudden increase takes place when the portion containing the granular secretion is reached. The cells lying towards the duct differ from those lying towards the acinar end of this portion. There is, however, no line of demarcation between them, the one gradually becoming changed into the other. In the centre of each cell is a clear body, pushing the nucleus and protoplasm to the outer portion of the cell. Towards the duct end in the centre of this clear substance is a darker portion continuous with the duct lumen. As the cells come to lie nearer the distal portion this central dark lumen becomes obliterated. This structure, though present in *Anopheles*, may be absent in *Culex*. In certain *Culex* another variation in the gland cells frequently occurs. The portion of the gland lying close to the duct, instead of being less in diameter, is greater. The cells composing this portion are columnar in shape, with centrally situated nuclei and no contained secretion.

In certain specimens it is not uncommon to find cells occupying a peripheral position and not approaching the lumen which contain a substance resembling the colloid-like secretion of the clear type of gland.

Changes after Feeding.—Very little change occurs in the glands after feeding. They are for the most part still quite full of secretion. Probably a very small amount only of secretion is used with each puncture.

The Ducts.—The intra-acinar ducts vary in *Culex* and *Anopheles*. In *Culex* they remain narrow and tubular throughout the entire length of the gland. In *Anopheles* they become large spaces in both types of acini but especially in the clear type. The duct is lined throughout by a clear homogeneous skeletal material which is continuous with a similar substance dividing the cells of the gland from one another. Into the duct the secretion-filled cell opens by means of a small opening.

The duct after leaving the acinus consists of a thick-walled tube with a central spiral thread resembling the spirals in the tracheæ. The wall is homogeneous but contains many nuclei.

The Malpighian Tubules.—The malpighian tubules are tubular bodies with cæcal ends which open into the hind gut. The cells are extremely large, being, next to the pericardial cells, the largest in the body. Each cell contains a large nucleus and contains numerous large granules which stain feebly with hæmatein but powerfully with Heidenhain's hæmatoxylin. Numerous fatty granules are also present. Each cell is wrapped round a central lumen, the cells being arranged alternately so that a zig-zag appearance is given in section. The inner portion of each cell is markedly striated, the lumen being thus bounded by a striated area. In relation with these tubules a large number of tracheæ and tracheal end cells exist.

In certain conditions the malpighian tubule cells may be found quite free from granules, though otherwise unchanged This change occurs in mosquitoes with large numbers of a flagellate organism (previously noted) in the rectum and hind gut

The Muscular System —The muscular fibres of the mosquito are without exception striated Those of the wings differ in structure very much from those of the limbs and body segments The muscle fibres of the alimentary canal are large fusiform cells with a single large nucleus with some surrounding protoplasm The muscle fibres in connexion with the heart are much branched

Many of the fibres contain a very marked sarcolemma and space between this latter and the fibre This space is usually seen occupied by extremely delicate branching threads which stain feebly with haematein

In the pupae there exist some large cells of peculiar nature in association with the sheaths of the muscle fibres

The structure of insect muscle is described in many works on histology and does not need repetition here

The Tracheal System —The larger tracheal vessels consist of a single layer of flattened cells with an inner chitinous layer In smaller tubes the cells embrace the entire vessel the nucleus frequently being bent around the lumen The cells of the tracheal vessels contain numerous small clear vacuoles (chitin formation) The chitinous lining possesses a thickening in the form of a spiral thread which may become unwound and lie stretched as a wavy thread in fresh preparations

The smaller tubes contain the spiral thread until they become from 2 to 5 μ in diameter. They then divide to form bundles of excessively minute air capillaries which enter among the tissue cells. The division into capillaries takes place in the substance of large branched cells situated at the termination of the tracheal vessels. The cells often appear cribriform in section from the number of air capillaries. These cribriform cells in connexion with the tracheal endings are well seen in the mid gut and malpighian tubules. They are however seen best of all in the undeveloped ovary of the newly hatched mosquito, which is extremely rich in bundles of capillary air tubes.

The Vascular System.—The dorsal vessel is a delicate walled tube composed of longitudinal and oblique fibres with a nucleated inner layer. The fibres may be traced directly from the terminations of the branched alary muscle fibres. The alary fibres break up into fibres which pass in close connexion with the large pericardial cells and eventually form (1) fibres passing into the dorsal vessel as longitudinal fibres, (2) fibres joining in an anastomosis in connexion with the floor of the dorsal vessel.

The pericardial cells are extremely large cells lying on either side of the dorsal vessel throughout its whole extent. They are by far the largest cells in the mosquito, varying from 30 μ to 50 μ in longitudinal diameter. They are elongate or pear-shape in form and contain several nuclei. The nuclei usually show signs of degeneration. The peripheral portion of the cell stains more deeply than the central portion, which contains the

nuclei and small stained granules There are a considerable number of masses of a light yellowish pigment resembling that found in the large visceral ganglia cells The fibres from the branches of the alary muscles pass over and around the pericardial cells to reach the dorsal vessel From their structure and situation the *pericardial cells* appear to be of the nature of ganglion cells (Fig 32)

The Fat body —The fat body, both where it occurs as a portion of the body wall and where it lies as free lobulated masses consists of cells containing numerous oil globules The cells are of considerable size and their borders may be frequently traced as polygonal areas The nuclei are oval in shape with a central mass of chromatin and chromatin threads Besides oil globules the cells contain granules staining with haematein, and minute droplets of a highly *refractile* dark substance which gives the appearance of pigment These droplets are larger in amount in old mosquitoes than in those freshly hatched (Fig 32)

The Nervous System —The ganglia of the ganglionic system consist of an outer portion of nerve cells and an inner portion of non medullated *nerve fibres* *Considerable complexity* exists in the larger ganglia especially the head ganglia

The ganglia of the visceral system differ greatly from those of the ganglionic system The ganglion cells are few in number and of large size They possess clear *reticular protoplasm* a little denser around the periphery than in the centre Around the inner margin of the denser peripheral portion small stained points are arranged In the centre a variable number of granules of yellowish pigment exist

The Reproductive System —Each ovary consists of a large number of follicular tubes whose lower ends open into the ovarian tube and whose upper ends terminate in a delicate supporting filament (terminal filament) The apex of the ovary is formed of a single follicular tube whose filament is attached to the fat body of the fourth segment

Around the whole ovary there is a delicate nucleated sheath

Each follicular tube contains one or more egg follicles in different stages of development In the freshly hatched mosquito each follicular tube contains an undeveloped egg follicle As this develops a second and a third undeveloped follicle appear above it which again undergo development into mature eggs The follicle at first consists of two to four large cells with large nuclei surrounded by a single layer of smaller epithelial cells (Fig 39)

The central cells then increase in size and number so that many very large cells are contained in the now enlarged follicle The surrounding epithelial cells also become larger and rapidly increase in number so as to form a layer of regular cubical cells surrounding the follicle The central cell nearest the ovarian tube is the ovum the rest are nurse cells and eventually disappear Both the ovum and the nurse cells increase greatly in size The nurse cells have clear protoplasm and extremely large nuclei which exhibit karyokinetic figures The ovum contains very numerous yolk granules which occupy the whole of its substance except a thin coating of granular protoplasm Still later this thin

external layer can only with difficulty be made out (Fig 39)

The nucleus of the ovum undergoes very pronounced changes It appears as an irregular mass, staining uniformly with nuclear stains This mass becomes more and more distorted and broken up and eventually disappears It may frequently however, be seen as irregular masses of staining material even in the mature egg A portion of the nucleus is seen very early to be separated off from the rest often surrounded by the latter This portion (female pronucleus) is small and difficult to detect in sections in the more mature ovum As the ovum increases still more rapidly in bulk, the nurse cells become crowded into the distal portion of the follicle and eventually disappear, so that, in the mature egg no trace of them is to be seen The epithelial layer surrounding the follicle becomes much flattened and forms eventually a covering to the egg (chorion) The outer portion of this covering (exochorion) is transparent, and marked with oblique parallel markings Over the proximal end *i e* the end lying towards the ovarian tube, the chorion forms a globular mass ornamented with rows of pits This is the micropylar apparatus through which the spermatozoa penetrate the ovum

Frequently in *Anopheles* a large portion or the whole of the adult ovum consists of a mass of sporozoa These consist of numerous small cysts each containing eight round or crescent shaped bodies each with a central chromatin spot (Fig 40)

The ovarian tube arises in the centre of the ovary and receives on all sides the follicular

tubes It is lined with a single layer of small cubical epithelium After passing out of the ovary, a considerable number of striated muscular fibres are arranged in a loose network around it and pass from it to surrounding structures There are also muscular fibres in the ovary itself in connexion with the ovarian tube and egg follicles

The spermatheca consists of a chitinous sac with large cells lying externally These resemble the cells of the cuticle and contain droplets They do not cover the whole of the surface of the spermatheca The contents of the spermatheca in the fertilized insect consist of a mass of spermatozoa which in the fresh state may be seen revolving with great rapidity within the sac The spermatozoa have a narrow slightly curved head and a long tail The duct of the spermatheca is narrow and thick walled and contains muscular fibres Certain large cells lie in connexion with the duct externally The mucus gland contains cells filled with secretion There are small nuclei in connexion with the intra acinar duct (Fig 39)

Chapter VIII

TO COLLECT AND PRESERVE MOSQUITOES

HOW TO COLLECT MOSQUITOES

Mosquitoes may be collected in two ways —

1. By capturing the adult flies
2. By breeding out from larvae and nymphae

1 Search in the daytime in houses, huts and out houses, at the base of large trees amidst brushwood, and other dark or shaded places *Anopheles* however are rarely caught except in huts and out houses They are especially fond of cow sheds and the darker portions of the eaves of huts

Some species of mosquitoes may be caught by sitting with a light near a white wall or suspended sheet or inside a tent, near a jungle or marsh *Culex* and *Taeniorhynchus* may be found sitting on the surface *just beyond* the brightly illuminated area *Anopheles* are rarely caught in this way but one species at least (*A barbirostris*) appears to be attracted by light and was caught by us on an illuminated sheet at night near swampy land

In searching for adult *Anopheles* as many places as possible should be examined as the distribution of some species is very local

If the captured insects appear to have fully matured ovaries, some of these should be placed in bottles as previously described (p 97) and allowed to lay their eggs

If care is taken to place only one species in a bottle the characters of the ovum may be noted in addition to the adult insect

Some of the ova should be placed in fresh water and an attempt made to determine the characters of the larva (p 73) when it has hatched out and is sufficiently grown

2 *Breeding out* —Full grown larvae and especially nymphae are collected These are collected from every possible source Scarcely any water will be found free from some form of mosquito larvae Even strongly brackish waters containing over one per cent of salt often contain large numbers

Examine water from the following sources —

(i) Domestic utensils cisterns tins pots calabashes in which there has been water for three or four days The larvae of *Stegomyia* *Culex* etc and only rarely *Anopheles* will be found

(ii) Cess pits pools full of decaying leaves etc sewage ditches Note larvae of certain species of *Culex* etc

(iii) Observe presence of the larvae of *Stegomyia* and *Culex* in the water which collects in the axils of banana leaves and other plants Also occasionally *Anopheles* in large collections of water of this kind

(iv) Puddles of all kinds with and without algae, ponds tanks swamps rice fields ditches canals, rivers streams lake margins and wells

scales are rubbed off, and that a crookedly mounted specimen is better than a rubbed one

5 *Push the pin steadily through the thorax*, so that it emerges as near the centre of the dorsum of the thorax as possible [Practise mounting by forcing the fine pin through without aid from the other hand]

6 Having transfixed the mosquito, force the point of the pin one millimetre beyond the back *by pressing it against the smooth surface of a cork* or tissue paper The pin should not be pushed through too far as it prevents the lens of the microscope being brought near enough for examination



Fig 41 Authors Method of Preserving Mosquitoes

7 Placing the disk against a cork, pass carefully through the edge a large entomological pin This is passed in the reverse direction to the fine pin Force three quarters of the length

of the large pin through the cardboard disk and then firmly press the point into the cork of a specimen tube so that when the tube is corked the mosquito is inside (Fig. 41).

In damp climates it may be necessary to carefully dry the tube and insect in a dessicator (over sulphuric acid or lime) or by placing in the sun or warm place to prevent mould. This however is but seldom required. Mites are rarely seen in insects preserved in tubes as described.

Write any information, *e.g.* locality, date or reference number *upon the outer surface of the cork* and on the edge of the cardboard disc. For transmission all that is necessary is to pack the tubes in wool in a box and send by post. Packed in this way they are far more secure than when mounted in the ordinary way in an entomological box. Mosquitoes for the British Museum should be addressed:

The Director,
The British Museum
(Natural History),
Cromwell Road, London, S.W.

Endeavour always to send both male and female, at least two of each, and what is of the greatest possible importance for the advance of our knowledge of mosquito classification, the careful description of ova and larvae.

N[illegible] If [illegible] it [illegible] to [illegible] mount mosquit[illegible] [illegible] placed between [illegible] paper [illegible] is far better than [illegible] method [illegible] by which treatment they [illegible]

To Mount Portions of Mosquitoes Permanently

1.—*Wings*.—Clip off one or both wings as near as possible to the thorax, so as to avoid cutting the base of the wing itself.

2. Allow the severed wing to fall in the centre of a clean glass slide. See that the dorsal surface of the wing is upwards.

3. Place one or two very minute drops of *thick* Canada balsam at some distance from the wing but within the area of a coverglass. This is merely to hold the coverglass firmly in place; the balsam must not be allowed to touch the wing.

4. Press a coverglass firmly down on the Canada balsam. If it is desired, the coverglass may be ringed with paraffin or some material which will not run by capillarity beneath the coverglass.

Legs.—Mount the legs of one side in order in a similar way.

Mount the palps and proboscis.

Mount (*a*) The male ungues.
(*b*) Scales from head, scutellum, etc.
(*c*) Wing denuded of scales.

In Canada balsam by placing a drop of balsam on these and mounting in the ordinary way.

Chapter XII

ANOPHELES: EXTERNAL ANATOMY OF THE IMAGO

The Head

The head is composed mainly of the two large compound eyes. These meet below and approach one another very closely above.

Parts of the head. The following are the usual names for the different regions of the head (Fig. 42):

1 The nape: the extreme back of the head.
2 The occiput: the portion behind the eyes.
3 The vertex: the space between the eyes.
4 The frons: the space in front of the eyes.
5 The genae: the side of the head below the eyes.

The frons is triangular in shape with one angle directed downwards. From the upper two angles arise the antennae and from the lower projects the clypeus lying over the base of the proboscis.

The Clypeus. Projects over the base of the proboscis as a prolongation of the frons. The character of the clypeus is of specific importance. It is

1 Hairy in *Culex*.
2 Scaly in *Stegomyia*.
3 Nude in *Joblotia*.

The Antennae —Consists of fourteen to sixteen segments, of which the basal one is large and globular. The plumose antennae of the male readily distinguishes it from the female.

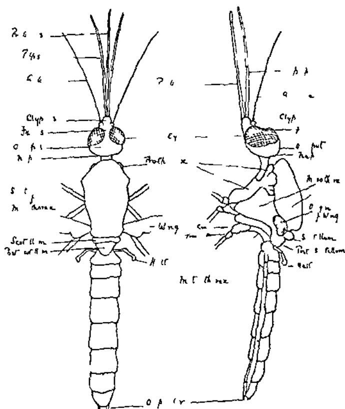


Fig 4 *External Anatomy of Female Anopheles*

The Proboscis —The proboscis consists of the very highly specialized mouth parts, ensheathed

in the lower lip or labium The proboscis consists of (Fig 43) —

1 The labium forming the sheath
2 The labrum and epipharynx or upper lip } forming the stylets
3 The hypopharynx or tongue
4 Two mandibles
5 Two maxillae
6 Two maxillary palps

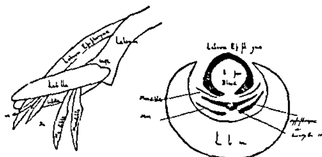


Fig 43 The Proboscis (Labium and Stylets) after NUTTALL *and* SHIPLEY *Right hand figure section of proboscis* *[illegible]*

The Labium — The labium forms the thick and scaly proboscis as usually seen On its dorsal surface it is hollowed out and in this hollow run as in a sheath the piercing mouth parts or stylets (Fig 43) The labium itself does not penetrate the skin but becomes sharply bent during the act of biting just as when a cane walking stick is pushed against the ground This may easily be seen if a mosquito is watched during the process of biting

The Labellae—Attached to the end of the labium by a hinge joint on either side are two leaf like processes, the labellae (Fig 43) It is through the angle made by the two labellae that the stylets pass as a billiard cue, between the first and second fingers (NUTTALL and SHIPLEY)

The labium proper stops short at the point of junction of the labellae but is continued on its upper surface as a blunt point covered with fine hairs (DUTTON) We may liken it to a pen continued on beyond the penholder, the junction of pen and penholder being the point at which the labellae are hinged on

Dutton's Membrane—The area between the end of the labium proper and the extreme tip is covered by an extremely thin membrane (DUTTON) In the act of biting, when the labellae are separated this membrane is somewhat stretched and applied to the skin

THE ESCAPE OF THE FILARIAL EMBRYO

It has been shewn by LOW and JAMES that the filarial embryo occurred in the proboscis, according to LOW among the stylets According to DUTTON the embryo lies really in the tissue of the fleshy labium moreover with its head at the level of the membrane described above and that it is by the rupture of this excessively thin membrane that the embryo escapes GRASSI and NOÈ think that the embryo escapes through the middle of the bent up labium through a rupture at this point but DUTTON'S *explanation seems* more likely

The *epipharynx* is the central tube through which the blood is sucked *Its point slopes off*

somewhat like the tip of a hypodermic needle In cross section it has the shape of an Ω the completion of the tube being formed by the apposition below of the hypopharynx The labrum is blended with the epipharynx but does not extend to the tip

The *hypopharynx* is a thin flat two edged lamella closely applied to the under surface of the epipharynx It is pierced by the salivary duct down which the salivary secretion and sporozoits pass The opening of the duct is continued as a groove reaching almost to the tip of the hypopharynx

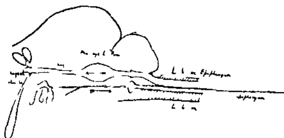


Fig 43x Showing ... Pharyngeal and Salivary Pumps ... the ...

The *mandibles* are very fine chitinous rods in cross section crescentic in shape At the tip of the mandibles are about thirty serration though in certain species of *Culex* these appear to be absent

The mandibles are closely applied to the sides of the epipharynx

The *maxillae* are stouter than the mandibles and fit around the outer side of these and the

hypopharynx They have about twelve serrations at the extremity coarser than those of the mandibles In some culices papillae replace the serrations

The Maxillary Palps —These lie upon either side and somewhat dorsally to the proboscis In the act of biting they take no part but are then separated from and lie at right angles to the proboscis Differences in the palpi are of both specific and generic importance

The expanded ends of the palpi in the male *Anopheles* are even more conspicuous than the plumose antennae

The Prothorax —The main portion of the thorax is mesothoracic anteriorly however, there is a collar like piece of chitin the prothorax To this are attached two moveable bodies the patagia

The prothorax is of importance in classification *e g* in the new genus of the *Anophelina Stethomyia* the prothoracic lobes are mammillated

The Mesothorax (Fig 42) —The *scutum* of the mesothorax forms the large globular mass of the thorax Behind the scutum, and just behind the origin of the wings is a transverse bar of chitin the *scutellum* Behind the scutellum is a convex triangular area extending as far as the first abdominal segment the *post scutellum* (Fig 42)

The scutellum and post scutellum are of importance in classification Thus the scutellum with its posterior border bristles is often of specific value whilst the post scullem may be—

1 Bare *Culex* and *Anopheles*
2 With hairs *Wyeomyia*
3 With scales and hairs *Joblotia*

THr Wɪɴc

The wings shew —

1 An interior straight, thick and strong border or costa

2 A posterior curved and thin border carrying a fringe

3 Two small folds at the base of the wing (squama and alula)

4 Nervures or veins

The *costa* in *Anopheles* is generally covered in part with white and in part with black scales (spotted)

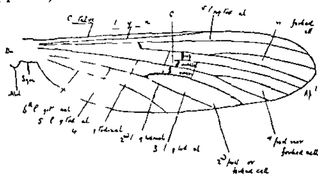


Fig 44 Wing of Anopheles —
Upper = Supernumerary cross vein
Inner = Inferior cross vein
Anterior fork cell = First forked cell
First posterior forked cell = Second posterior cell
Second posterior forked cell = Anal cell

The *fringe* in *Anopheles* has most frequently light and darker portions the number and position of which are of specific importance (Fig 51)

The *squama* and *alula* are shewn in the figure. They are of no great importance (Fig 44)

The Venures —The nervuration of the wing is of considerable importance Several nomenclatures are in use That used in the accompanying diagram is, however, the simplest (Fig 44)

In classification, the relative position of the apices of the two forked cells are frequently used Also the relative positions of the point where the auxiliary vein cuts the costal vein, and the point where the fifth vein cuts the posterior margin As a rule, the position of this first point is much nearer the base than that of the second point, but in a few instances *e g M sinensis*, they almost coincide

Also the positions of the upper, middle, and cross veins It will be found, however, that even in the same species there is no constancy in these latter and they can hardly be given as of specific importance as has been done DÖNITZ has made the same criticism, and indeed, finds that the position in each wing of the same mosquito may be different

THE LEGS

These consist of the following segments —

1 Coxa and trochanter Small pieces at the origin of the legs (Fig 42)

2 Femur

3 Tibia

4 Tarsus consisting of five segments, the last of which carries the claw or ungues



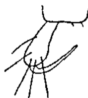
Fig 45 *Fore Ungues of M Funesta (♂) the larger Uniserrate*
Fore Ungues of M Rossii (♂) the larger Biserrate
(*After* THEOBALD)

The Ungues.—The ungues vary in the male and female and in the different legs. They may be simple, uniserrated or biserrated (rarely triserrated) (Fig. 45). They are of specific value (THEOBALD).

THE ABDOMEN

The abdomen consists of nine segments. To the ninth segment are attached the genitalia.

The genitalia are variously shaped lobed appendages. In the male they are provided at their free end with claspers. The claspers in the male are of specific value (Fig. 46).



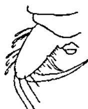


Fig. 46. Male Genitalia.

LITERATURE

1. *Reports to the Malaria Committee of the Royal Society,* Series IV. *The Anatomy and Histology of the Adult Female Mosquito* by S. R. Christophers, M.B. Vict. (with 6 plates). Price 3s. 6d.

2. Nuttall and Shipley. *Journal of Hygiene,* Vol. I, No. 1; Vol. I, No. 4; Vol. III, No. . *The Anatomy of the Larva, Pupa and Imago.* Well illustrated.

3. *Liverpool School of Tropical Medicine.* Memoir IV, Part II. Filariasis, including a minute study of the proboscis of the mosquito. Many illustrations. (Price 1s. 6d.)

Chapter XV

CLASSIFICATION AND IDENTIFICATION OF THE CULICIDAE

SCALES

THEOBALD has attached to scale structure the greatest importance from the point of view of generic and specific classification Hence it is necessary to consider somewhat in detail these structures THEOBALD gives the following —

Head Scales — Three forms of scale occur (Fig 47)

1 Narrow curved scales
2 Upright forked scales
3 Flat scales overlying one another like the tiles of a roof

No 1, 2 and 3 scales found, *e g*, *Culex*
No 2 and 3 scales only, found = *Stegomyia*
No 3 scales only found = *Megarhinus*
Toxorhynchites

Thoracic Scales — THEOBALD describes five forms (Fig 47)

1 *Narrow Hair like Curved Scales* — They often form a dense feltwork over the mesothorax

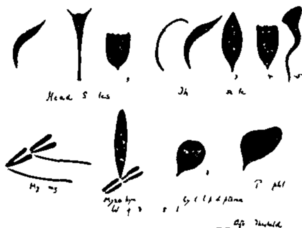
2 *Narrow Curved Scales* — They may occur all over the mesothorax and scutellum, or at the sides of the scutum and in front of the scutellum

3 *Spindle Shaped Scales* — These lie scattered about and never form a complete covering

4 *Flat Scales* like those on the head They cover the scutellum in *Stegomyia* whereas in *Culex* the scutellar scales are of the narrow curved type

5 *Long Twisted Scales* —Characteristic of *Mucidus* a genus of mouldy looking mosquitoes

Abdominal Scales —The scales covering the abdomen in all *Culicidae* except the *Anophelina* are overlapping flat scales In the *Anophelina* they are not found except to some extent in *Myzorhynchus* and *Nyssorhynchus* In the genus *Aldrichia* however of *Anophelina* the abdomen is covered with flat scales as in *Culex*



[Fig 41 Varieties of Scales (after Theobald)]
Fan plates = Mansonia

The *Culicidae* are divided into the following sub families based mainly upon the length of the palpi in male and female

1 Palpi long in both sexes as long as the proboscis in the female *Anophelina*

2 Palpi long in both sexes, shorter than the proboscis in the female *Megarhinina*

3 Palpi short in female long in male—*Culicina*

4 *Palpi short in female, long in male* Post scutellum with hairs (chaetae) and scales *Joblotina* [= *Trichoprosopina*

5 Seven (not six) longitudinal veins with scales *Heptaphlebomyina*

6 Palpi very short in female and male—*Aedeomyina*

7 Proboscis short not formed for piercing *Corethrina*

Wing Scales—Scales clothe the veins, except the cross veins Flat scales are arranged in a double row along each vein

Many, or in some species all, of the veins have also lateral scales

The lateral scales are very variable in shape, *e g*,

1 In *Mansonia* they are broad asymmetrical flat scales

2 In *Aedomyia* the scales are similar

3 In *Mucidus* the wing scales are quite characteristic, being pyriform or inflated and half dark, half white

4 In *Megarhinus* the scales may be azure green or blue

The Wing Fringes consist of—

1 Long narrow pointed scales attached to the edge of the wing by a narrow stalk

2 Smaller scales similar in shape

3 Border scales Small flat scales

Leg Scales—The legs are covered with flat scales in nearly all *Culices*

1 In *Sabethes* the scales are hair like and occur in tufts

2 In *Mucidus Psorophora*, the scales are elongated and project from the legs

The sub family *Anophelina* contains as we shall see seven genera the *Culicina* fifteen and the *Aedeomyina* twenty When we consider further the large number of species in some of these genera, *e g* *Culex* it is impossible to attempt here to describe each mosquito however briefly Considering the great importance however of the *Anophelina*, we shall attempt to give the characteristic specific points for each of the species as an aid to a detailed examination by means of THEOBALD'S monograph With regard to the other sub families we shall attempt only to give characteristics of each genus

SUB FAMILY ANOPHELINA (*vide* next Chapter)

SUB FAMILY MEGARHININA

Genus 1 *Megarhinus* – First sub marginal cell much smaller than second posterior cell Palpi

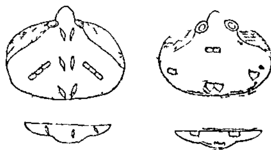


Fig 48 Head and Scutellar Scales of Aedes (left) Megarhinus (right)

five jointed in ♀ (*M purpureus* only four) Readily recognized (i) by their large size often called elephant mosquitoes (ii) by their brilliant metallic colours (iii) by a caudal tuft of hairs on each side of the abdomen (iv) by the long and curved proboscis (v) head is clothed with flat scales only (Fig 48)

They may be found resting on the trunk of trees in the forest, also in houses in the bush Species about six

Genus 2 *Toxorhynchites* —Palpi much shorter than proboscis in ♀ three jointed Supernumerary cross vein nearer the apex of the wing than the mid cross vein Species four

Sub Family Culicinae

First sub marginal cell equal to or longer than the second posterior cell

Genus 1 *Janthinosoma* —Hind legs densely scaled giving a characteristic appearance Species five

Genus 2 *Psorophora* —Characterized by (i) great length of ♂ palpi five jointed (ii) densely long scaled legs (iii) posterior cross vein a little nearer the base than the mid (iv) proboscis curved in ♀ Species, four

Genus 3 *Mucidus* —Easily recognized by their curious mouldy appearance Posterior cross vein nearer apex of wing than mid Wing scales large pyriform parti coloured Head and thoracic scales long and twisted expanded at the apex Legs densely scaled with projecting scales Species, five

Genus 4 *Desmodea* = *Armigeres*.—Head flat scales a few upright forked. Differs from *Stegomyia* (1) is longer with unbanded tarsi and abdomen. ♂ palpi untufted. ♀ palpi very pointed and provided with bristles only. Species two.

Genus 5. *Stegomyia*.—Head completely clothed with broad flat scales (Fig. 49) and a few upright forked. Palpi four jointed in the ♀ five jointed in the ♂. *Scutellar scales flat*, mostly black and white mosquitoes with banded legs and abdomen. Species eighteen.

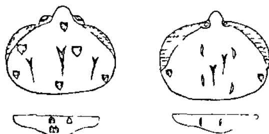


Fig. 49.—Head and Scutellar Scales of Stegomyia (left) and Culex (right).

S. *fasciata*. Transmits yellow fever.

1. Tarsi *basally* banded white.
2. Proboscis unbanded.
3. Thorax. A pure white broad curved band on each side and two median pale parallel lines.
4. Ungue of ♀ toothed.

Genus 6. *Theobaldia*. Palpi in ♂ clubbed as in the *Anophelina*. Palpi in ♀ five jointed, apical joint mammilliform; wings in both sexes densely

\

scaled collected into spots, thus forming a *spotted wing* group of mosquitoes Species, five

Genus 7 *Lutzia*—Resembles *Theobaldia*

Palpi in ♀ three jointed, apical joint not mammilliform

Palpi in ♂ not clubbed, three jointed

Wings spotted by scales similar to those of *Taeniorhynchus* Species, one

Genus 8 *Culex*—Head scales *narrow curved and upright forked* laterally flat scales (Fig 49) Palpi in ♀ three jointed Third palpal joint usually is long or longer than the other two

Wing first sub marginal cell longer and narrower than the second posterior Posterior cross vein nearer the base than the mid wing veins Scales small lateral ones linear

Scutellum *narrow curved or spindle scales* (Fig 49)

C mimeticus has spotted wings Species very numerous

Genus 9 *Gilesia*—Related to *Culex* and *Stegomyia*

(i) Scutellum *with small flat scales*, some spindle scales

(ii) Head broad flat, spindle scales

(iii) Basal joint of antennae hairy and scaly

(iv) Claws short and thick with a blunt tooth

(v) Wing scales like those of *Taeniorhynchus* Species one

Genus 10 *Lasioconops*—

(i) Head scales as in *Culex* Basal joint of antennae, a few scales

(ii) Abdomen *large projecting flat scales with deeply dentate apices* giving these mosquitoes a ragged appearance Species one

Genus 11 *Melanoconion* —Distinguished from *Culex* by the dense broad scales on the costa and apex and by the black spine like scales along the upper border Small dark mosquitoes Species six

Genus 12 *Grabhamia* Allied to *Culex* and *Taeniorhynchus* Palpi in ♀ four jointed Apical joint minute Penultimate long and thick Wing scales not so long or dense as in *Taeniorhynchus* Scales mottled Wings short and stumpy Legs mottled and spotted Species ten

Genus 13 *Acartomyia* Allied to *Culex* and *Grabhamia* Distinguished from *Grabhamia* by having *flat irregularly disposed scales* all over the head from *Culex* in the ♂ palpi Two terminal segments and the apex of the antipenultimate swollen Terminal segment club shaped Ragged appearance of head well marked Species one

Genus 14 *Taeniorhynchus* Palpi five jointed in ♂ the fifth segment minute Characterized by the wing scales They are *thick elongated scales ending with a broad sloping concavity or blunt point* median linear scales often absent proboscis usually banded Species about sixteen

Genus 15 *Mansonia* *Panoplites* Palpi four jointed in ♂ more than one third the length of the proboscis Characterized by *wings densely scaled along the veins with broad asymmetrical flat scales* No *median scale* The genus resembles *Aedeomyia* but the palpi in the ♂ are long in members of this genus short in the *Aedeomyia* Species eight

Sub Family Joblotina

Genus 1 *Joblotina*.—Metanotum (= Post scutellum) *with a tuft of chaetae and with flat scales*. Clypeus and base of antennae bristly. Second long vein carried nearly to the base of the wing. Second posterior fork cell (anal cell) very large. Mid cross vein nearer the apex than the anterior (supernumerary). Wings densely scaled, scales shorter than in *Taeniorhynchus*. Species one.

Sub Family Heptaphlebomyina

Genus 1 *Heptaphlebomyia*.—Like *Culex* but has a distinct seventh vein. Species one.

Sub Family Aedeomyina

Genus 1 *Deinocerites* = [*Brachiomyia*].—Characterized by the ♀ antennae. Much longer than the proboscis. *Second segment as long as the three terminal segments*. Antennae scaled. Antennae in ♂ pilose and longer than the whole body. Species two.

Genus 2 *Finlaya*.—Three ventral abdominal scale tufts. Scutellum four median bristles. Wing scales large and broad, pyriform. Species, two.

Genus 3 *Aedes*.—Head narrow curved scales forming a *broad median line only*. Other scales flat. Scutellum *narrow curved scales*, six bristles. Palpi in ♀, four segments, apical segment minute, mammilliform. Traces of a fifth segment. Species two (Fig. 48).

Genus 4 *Howardina*.—Resembles *Aedes*, but scutellum has only four bristles. Palpi, four

segments apical, one minute not mammilliform Species two

Genus 5 *Aedimorphus* —Head mostly flat scales narrow curved behind Scutellum *flat scales* eight (?) bristles Has no flat thoracic scales as *Uranotaenia* Species one

Genus 6 *Skusea* —Head *flat scales only* Anterior and posterior forked cells densely scaled Palpi in ♀, three segments Scutellum six bristles and narrow curved scales Species three

Genus 7 *Verrallina* —Head as in *Skusea* Palpi two segments only (trace of a third) apical segment large Scutellum four bristles and narrow curved scales Species three

Genus 8 *Ficalbia* Intermediate between last two and next genus Head scales *no narrow curved* almost entirely flat Scutellum *flat scales* as in *Uranotaenia* but *thoracic scales narrow curved* Palpi two segments Species two

Genus 9 *Uranotaenia* Head *flat scales* upright forked may or may not be present Scutellum *flat scales* Thorax *narrow curved and flat scales* Wings small forked cells Metallic scales at the base of the wings Related to *Aedes* but more brilliant (metallic) and stouter mosquitoes Species fourteen

Genus 10 *Myomyia* —Resembles *Uranotaenia* Has no flat scutellar or thoracic scales Forked cells larger than *Uranotaenia* No metallic scales at the base of the wings Species two

Genus 11 *Aedeomyia* Allied to *Aedes* Distinguished by (i) head scales upright fan shaped clypeus scaly (ii) thorax broad flat spindle scales (iii) scutellum broad flat scales (iv) legs

densely scaled (v) wings, densely scaled as in *Mansonia* also with long lateral scales Species, three

Genus 12 *Haemagogus*—Related to *Aedes*, but palpi five segments Head covered with flat scales Brilliant metallic (blue) mosquitoes Species, two

Genus 13 *Wyeomyia*—*Chaetae on the post scutellum* Head flat scales Thorax, spindle and flat scales Scutellum flat scales Palpi short *Proboscis not as long as whole body* Species two

Genus 14 *Phoniomyia*—Resembles *Wyeomyia* but distinguished by (i) wing scales broad lateral scales as in *Taeniorhynchus* (ii) proboscis longer than the whole body Species, two

Genus 15 *Dendromyia*—Resembles *Wyeomyia* distinguished by (i) scutellar scales small flat rounded apically (ii) wings more densely scaled than in *Phoniomyia* scales *Taeniorhynchus* like (iii) proboscis moderately long Species, five

Genus 16 *Runchomyia*—Allied to *Dendromyia* Characterized by (i) frons projecting as a blunt spine (ii) *proboscis as long as the body in* ♀ (iii) ventral apical tuft of bristles (iv) wings covered with rather broad scales Species one

Genus 17 *Sabethes*—Distinguished from *Wyeomyia* by the *asymmetrical wing scales* One or more *legs with dense paddle like structures* in both sexes Mid cross vein nearer the apex than the anterior Posterior nearer the apex than the mid in the ♂ Third long vein carried through into the basal cell Brilliant metallic mosquitoes Species four

Genus 18 *Sabethoides*—Closely resembles *Sabethes* Distinguished (i) by much smaller palpi (ii) unpaddled legs Species one

Genus 19 *Goeldia*—Post scutellum with *chaetae and scales* Wing scales as in *Runchomyia* dense elongated Wing venation as in *Culex* Proboscis short and thick not as long as body Palpi in ♂ one third length of the proboscis In ♀ quite short Species one

Genus 20 *Limatus* Characterized by the proboscis bent in the middle densely scaled at the bend Species one

Sub Family Corethrinæ

Genus 1 *Corethra*—First tarsal segment longer than the second tarsal

Genus 2 *Mochlonyx*—First tarsal segment shorter than the first tarsal

LITERATURE

1 *Monograph of the Culicidae* Vol I III F V Theobald *Brit Mu Nat Hist* The data in this chapter has been taken almost entirely from Theobald's work

Chapter XVI

THE CLASSIFICATION AND IDENTIFICATION OF THE ANOPHELINA

It is by no means an easy matter to fix definitely the *species* of an *Anopheles*, and yet the identification of species is essential in connexion with malarial studies. It does not suffice merely to ascertain that *Anopheles* are present in any given locality but it must be clearly made out what the *species* are. It is, indeed, only by accurately observing the relation of *Anopheles* to malaria that we can hope for the explanation of many of the difficulties surrounding the anomalous distribution of endemic malaria. In a later chapter the extreme importance of the species of *Anopheles* in this connexion will be evident. In the description of the habits of *Anopheles*, their breeding places, occurrence apart from man, etc., it is no longer sufficient to ascribe these to the whole genus but they must be ascribed to the actual species involved. The study of the *Anophele* from the point of view of classification and identification is therefore of importance.

CLASSIFICATION OF ANOPHELINA

THEOBALD has sub-divided the genus *Anopheles* into ten new genera. These genera comprise

over seventy eight different species. The assigning of an *Anopheles* (old sense) to its proper genus simplifies therefore very much the ultimate determination of the particular species. [illegible]'s classification is the following:—

Thorax and abdomen with hair-like curved scales	[illegible] head scales	Wing scales [illegible] lanceolate	*Anopheles*
		Wing scales mostly long and narrow	*Myzomyia*
		Wing scales partly large, inflated	*Cycloleppteron*
	[illegible] head scales	Wing scales [illegible] lanceolate	*Stethomyia*
Thorax with [illegible] narrow curved [illegible] scales, abdomen [illegible]	Wing scales small lanceolate [illegible]		*[illegible]*
Thorax with [illegible] curved scales [illegible] narrow curved [illegible] front, abdomen with [illegible] [illegible] ventral [illegible]			*[illegible]*
Thorax with hair-like curved scales, abdominal scales [illegible] [illegible] tuft [illegible]			*[illegible]*

Thorax and abdomen with true scales	Abdominal scales as lateral tufts and dorsal patches of small flat scales thoracic narrow curved or spindle shaped	*Nyssorhynchus*
	Abdomen nearly completely scaled with irregular scales and with lateral tufts	*Cellia*
	Abdomen completely scaled with large flat scales as in *Culex*	*Aldrichia*

These features are shewn in the accompanying diagram (Fig 50) The thoracic scales in *Cyclolepidopteron* are not sufficiently hair like (THEOBALD)

Place the mounted or unmounted mosquito under a low power of the microscope, and determine carefully the characters of the hairs or scales on the head, thorax and abdomen By this means the *Anophelete* is assigned to its proper genus

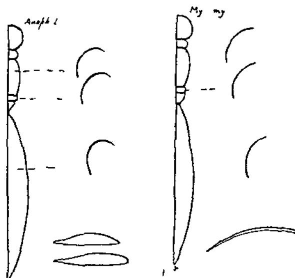




FIG. 5[illegible]—*Contd.* Th[illegible] Scutellar and [illegible] S[illegible]les of the [illegible] (after THEOBALD)

THE DIFFERENTIATION OF SPECIES

Many features are of value in determining the species

1 *The Wings* —

(*a*) They may shew areas of dark scales on the costa, auxiliary and first longitudinal veins producing the main spots of the wing

(*b*) Small areas of scales on the third to sixth long veins less dark and distinct than the large costal spots

(*c*) Pale areas on the wing fringe

(*a*) The markings on the wing are fairly constant in each species but variations occur so that the spot may be longer or shorter giving the wing a darker or lighter aspect in the same species Thus in A *Stephensi* the following variations in the second costal spot may be encountered (Fig 51). Especially does this variation occur in the wing of males The costal spots may also be confluent They may depart *from their typical shape as is frequently seen in the T spot* of M *Rossii* Certain general types of wing can be recognized —

(i) Wing almost entirely covered with black scales as in the genus *Myzorhynchus*
(ii) Wings only slightly spotted on the costa or wing field *e g* in the genus *Anopheles*
(iii) Wings with well marked discrete spots (*a*) brownish wings as in *Myzomyia* (*b*) black wings as in *Nyssorhynchus*

(*b*) The smaller spots on the wing field along the course of the veins are also useful for determining species Thus *M leucophyrus* has six spots on the sixth long vein while *M elegans* has only four The extent to which the third longitudinal vein is scaled is also of specific importance (Fig 5)

(*c*) The wing fringe has at the points where the long veins cut the margin a variable number of light areas Thus *A punctipennis* has only one *pale area* while *A pseudopunctipennis* has many Another example of this means of distinguishing species is given in the figure (Fig 51)

2 *Leg Markings* —

(*a*) Uniformly coloured as in the second division of *Myzomyia*

(*b*) Speckled or banded chiefly in the genera *Nyssorhynchus* and *Cellia* The banding of the legs is of great importance in distinguishing the species (Fig 53) thus (1) banded tarsi e g N *Maculata* (2) tarsi pure white e g N *fuliginosus* N *Jamesii*

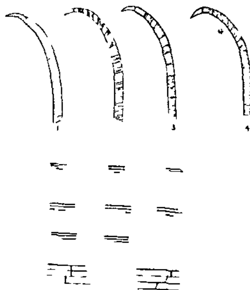


Fig 51 Wing fringes of (1) M Rhodesiensis ( ) M Funestus (3) M l i t ni (4) M Culicifacies
Variations in Wing Spots of M Rossii N Stephensi and I C talis
Variations of C s teins of M Rossii

THE DIFFERENTIATION OF SPECIES

Many features are of value in determining the species

1 *The Wings* —

(*a*) They may shew areas of dark scales on the costa auxiliary, and first longitudinal veins producing the main spots of the wing

(*b*) Small areas of scales on the third to sixth long veins less dark and distinct than the large costal spots

(*c*) Pale areas on the wing fringe

(*a*) The markings on the wing are fairly constant in each species but variations occur so that the spot may be longer or shorter giving the wing a darker or lighter aspect in the same species Thus in N *Stephensi* the following variations in the second costal spot may be encountered (Fig 51) Especially does this variation occur in the wing of males The costal spots may also be confluent They may depart *from their typical shape as is frequently seen in the* T spot of M *Rossii* Certain general types of wing can be recognized —

- (i) Wings almost entirely covered with black scales as in the genus *Myzorhynchus*
- (ii) Wings only slightly spotted on the costa or wing field *e g* in the genus *Anopheles*
- (iii) Wings with well marked discrete spots (*a*) *brownish* wings as in *Myomyia* (*b*) black wings as in *Nyssorhynchus*

(*b*) *The smaller spots on the wing field* along the course of the veins are also useful for determining species Thus *M leucophyrus* has six spots on the sixth long vein while *M elegans* has only four The extent to which the third longitudinal vein is scaled is also of specific importance (Fig 5)

(*c*) The wing fringe has at the points where the long veins cut the margin a variable number of *light* areas Thus *A punctipennis* has only one pale area while *A pseudopunctipennis* has many Another example of this means of distinguishing species is given in the figure (Fig 51)

2 *Leg Markings* —

(*a*) Uniformly coloured as in the second division of *Myzomyia*

(*b*) Speckled or banded chiefly in the genera *Nyssorhynchus* and *Cellia* The banding of the legs is of great importance in distinguishing the species (Fig 53) thus (1) banded tarsi *e g* *N Maculata* (2) tarsi pure white *e g* *N fuliginosus* *N Jamesii*

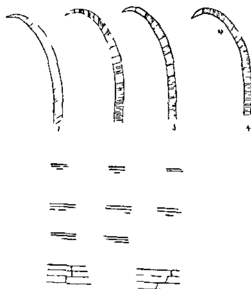


Fig 51 *Wing fringes f* (1) *M Rhodesiensis* ( ) *M Funestus* (3) *M List ni* (4) *M Culicifa ies*
Variations in Wing Spots of M Rossii N St phensi and P C stalis
Variati ns of Cross veins f M Rossii

3 *Palpi* —Similarly the bands or collection of white scales on the palpi is a convenient means of separating the members of a particular genus (Figs 52 53) It should not be forgotten that in these two characteristics there is a certain amount of variation possibly seasonal and a slight difference in the bands on the palpi and legs is not sufficient in itself to constitute a difference in species

Other characteristics that are useful for the determination of species are the male genitalia and the character of the ungues in the male whether having one or more teeth The position of the cross veins has also been used but this is so variable in the same species that it has little value

Characters of the Larvae and Ova —In the *Anophelina*, as in the rest of the *Culicidae* this is a most important means of differentiation Mosquitoes that otherwise are almost indistinguishable are readily separated by their larvae being different

One precaution must be taken It must be quite certain that it is the larva of the mosquito in question that is being examined The easiest way to make sure of this is to carefully examine the larva first, and then to hatch out the mosquito and then examine it The examination of the larvae is considered later

Genus 1 *Anopheles* —Wings unspotted or slightly spotted Larva clypeal hairs branched Ova type 1 Mosquitoes not particularly domestic in habits *Mostly belong to temperate climes* or hill districts

A Bifurcatus transmits malaria (in Italy) Zygotes have been found in *A maculipennis* in Spain but experiments with this species in England have failed though successful on the continent They may be divided up in the following way —

Costa Uniform Wings spotted

1 *A Maculipennis* —Wing with four spots apex of first tarsal joint spotted Europe

2 *A Crucians* —White spots on brown veins Three black spots on sixth vein Costa uniformly dark tarsi unbanded North America

Costa Spotted

3 *A Punctipennis* —Two yellow spots one at the apex the second on the apical third One fringe spot North America

4 *A Pseudo Punctipennis* —Wings as in previous species but wing fringe with several yellow spots North America

5 *A Gigas* —Costa two large black spots Length five to six mm A large hill species India

6 *A Lindesayii* —Costa black apical white spot Femora have a characteristic broad median white band A hill species India

Wings Unspotted

7 *A Bifurcatus* —Thorax Golden hairs arranged so as to leave two broad bare lines on the front *Abdominal hairs golden* Europe

A Walkeri is regarded by THEOBALD as identical with this

8 *A Algeriensis* —Abdominal hairs dull brown First forked cell shorter than in *A Bifurcatus*

9. *A. Nigripes.*—A black mosquito. No bands on tarsus. Europe, America.

10. *A. Immaculatus.*—Ash grey in colour. Slight apical bandings to tarsi. Palpi and proboscis lighter at apex. Ennur, Madras. A single species only found by us.

11. *A. Aitkeni.*—Uniformly dark. No markings on palpi or legs. Bombay Presidency.

12. *A. Stigmaticus.*—Light brown. Australia.

13. *A. Philippinensis.*

Genus *Myzomyia.*—To this genus belong those species which are associated in the tropics with the most severe endemic malaria, *e.g. M. Funesta* in Africa and *M. Listoni* and *M. culicifacies* in India. The group includes, however, several species one at least of which has, as far as our knowledge extends, no power of transmitting malaria in nature, viz. *M. Rossii.*

The malaria transmitters form a natural group: they are small dark mosquitoes, with unbanded legs and they breed in fresh natural waters, *e.g.* streams, river beds, etc., whereas we also have in the group domestic mosquitoes, *i.e.* those that breed in foul pools about houses. *M. Rossii* is the type of this class.

Whether in this genus any others than the three mentioned above convey malaria there are at present no facts to shew, and the larval characters of only the Indian species are at present known.

The type species is *M. Funesta* which is a typical spring and fresh water breeder. It is noteworthy that *M. Funesta* is associated with a higher malarial endemicity than *P. Costalis* which is a typical domestic mosquito breeding in foul pools.

Group I

Small dark mosquitoes breeding in natural waters

1 *M Funesta*—Costa six white spots Basal spots with pale interruption Wing fringe pale spots at ends of all the veins except sixth Palpi three bands the basal one further from the middle one than the apical A variable species third long vein may be dark Resembles *Listoni* and *Rhodesiensis* (Fig 51)

2 *M Listoni*—*Third long vein light* Wing fringe four or more light spots (Fig 51) Palpi two broad apical bands further apart than in *Funestus* one narrow basal Basal portion of costa uniformly black (characteristic) Attitude *Anopheles* like Associated with high endemic index in the Duars Bengal Larval characters antennae with simple hair Clypeal hairs simple Palmate hair on thorax and on all abdominal segments

3 *M Aconita* —*Aconita* = unspeckled be cause at the commencement of the third long vein the usual dark spot is absent Palpi four bands Costa four spots light interruption in basal spot Fringe several pale areas Anterior forked cell much longer and narrower than posterior Differs from *Listoni* in palpi Sumatra Java

4 *M Culicifacies*—*Third longitudinal vein dark* Wing fringe three spots at most Palpi three equal band two at the joints one at the apex *Attitude Culex like* Associated with high endemic index of malaria in the Punjab and Madras Larval characters as in *Listoni* Ova floats do not touch margin of upper surface (Type 1) (Fig 51)

5 *M Leptomeres*—Base of first long vein white Anterior forked cell much longer and narrower than posterior Costa, two spots thus differing from *Hebes* Fringe pale areas at all the veins

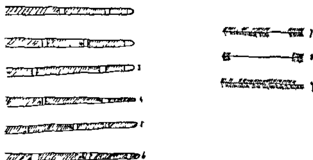


Fig 5
Palpi of M Funesta (1) M Listoni ( ) M Culicifacies (3) M Rhodesiensis (4) M Hispaniola (5) M Turkhudi (6) Third Long Veins of M Funesta (7) M Listoni (8) M Culicifacies (9)

6 *M Hebes*—*Hebes* = inconspicuous a small species resembling *Rhodesiensis* Wing costa four spots wing fringe, seven light areas Vein six one long spot Palpi first and second segments covered with white scales End of third segment is dark fourth segment quite white Distinguished from *Rhodesiensis* by palpi and wing fringe East Africa

Group II

Larger species than the above Generally lighter Wings not so covered with dark scales

Habits Domestic species breeding in foul puddles etc near houses

Larval characters (*M Rossii*) Antenna without branched lateral hairs Clypeal hairs simple Palmate hairs second to seventh segments and often rudimentary hairs on thorax and first and second abdominal segments Ova (type 2) i e floats touching margin of anterior surface

7 *M Albirostris* —Characterized by the banded proboscis pale scaled to about half its length *N deceptor* has also a banded proboscis Malaysia Length two to five mm

8 *M Longipalpis* —Palpi long thin three narrow white rings wing costa black four almost equal yellow spots wings mostly brown scaled hind legs only banded narrow apical and basal yellow bands British Central Africa Length three mm

9 *M Ludlowii* —Palpi apex broad white band a second small one close to it a third basal band Wing costa four large spots one or two small basal Legs femora tibiae and metatarsi especially in hind legs *spotted* with yellow Tarsi broad apical and basal pale banding especially in hind leg Philippine Isles Length four to five mm

10 *M Rossii* —Probably = *A Vagus* (DÖNITZ) Sumatra Palpi somewhat like those of *M Ludlowii* but easily distinguished the apical white band is broader the second band is much nearer the base than in *M Ludlowii* so that the black area between is longer Wings four spots and some basal spots The second large spot has the characteristic *T shape* but is very variable Tarsi slight pale apical and basal bands to some of tarsi India Malaysia

11 M *Lutzii* —Characterized by the linear ornamentation on the thorax, and marked bands (five in number) on the fore and mid metatarsi Wings three distinct pale spots, two smaller ones 3 to 3 5 mm Rio de Janeiro

12 *M Elegans* —Possibly = N *Leucophyrus* (Dönitz) Leukos = white sphyron = ankle joint Palpi four white bands Wing costa four large black scaled areas, three small Wing fringe six pale interruptions Legs speckled with white scales Femora and tibiae speckled in hind legs Characterized by a large tibio metatarsal band on the hind legs Resembles N *Stephensi* differs in the palpi has four not three spots on the sixth longitudinal vein Differs from N *Leucophyrus* in having four not six spots on the sixth vein Possibly belongs to *Nyssorhynchus* genus India

13 *M tessellatum* —Costa four large four small spots Fore tarsi apically and basally banded Mid and hind tarsi apically only Thorax two dark spots in front and 1 dark area near the scutellum Malay

14 *M punctulatus* —Costa four large spots and numerous dark and white spots Malay closely resembles the former

15 *M Leucophyrus* —Related to the two former Distinguished by prominent tibio metatarsal band and by the prominent median dark spot on costa Sumatra

16 *M Impunctus* Costa four small dark spots Fringe spotted Sixth vein three spots Relationship doubtful not fully described Egypt

Group III

Medium size dark mosquitoes. Apex of palpi black.

17. *M. Turkhudi.*—Palpi *apices black*, the band not so broad as in Hispaniola; third long vein mostly dark, but varies; pale interruption in basal costal spot. India. Larvae resemble *Culex*. Ova very peculiar, type 3 (*vide* p. 22).

18. *M. Hispaniola* (Theo.). Spain. Third longitudinal vein mostly pale yellow, except at the base and apex. Wing fringe with spots except where lower branch of fifth and sixth join the costa. Basal portion of costa uniformly black.

19. *M. Rhodesiensis* (Theo.). Rhodesia.—Third longitudinal vein dark. Palps with only two conspicuous bands. The palpi are much longer and thinner than in *M. funestus*. The veins are all dusky scaled. Base of the costa black. In *M. funestus* there is a white interruption. Wings: costa three small white spots and a yellow apical spot. *Fringe unspotted* except an apical spot (Fig. 51).

Genus *Cycloleppteron*.—Wings with numerous large inflated scales collected in patches or irregularly disposed.

Larval Characters (Theobald).—Antennae without lateral branched hair. Clypeal hairs simple. Palmate hairs six pairs. Lanceolate.

1. *C. Grabhamii.* Palpi unbanded. Jamaica.
2. *C. Mediopunctatus.*—Palpi banded black and gold. Brazil.

Genus *Stethomyia* (στηθος = breast).—Head with a median patch of flat scales. Palpi very thin.

1. *S. nimba.* British Guiana, S. America.

1 *A Maculipes* — Hind and mid legs much banded and speckled Almost certainly transmits malaria (LUTZ)

Genus *Myzorhynchus* — μυζω to suck ρυγχος proboscis

Palpi densely scaled in the ♀ also the proboscis These are wild mosquitoes found in situations remote from the dwellings of man They breed in swamp and large bodies of water especially those containing weeds They do not usually frequent houses *M Sinensis* is, however, attracted by light They feed readily on human blood when occasion offers

Larval Characters — They occur singly throughout large masses of water They have a peculiar stiff and stick like appearance, but they also exhibit curious attitudes sharp bends as they lie on the water Clypeal hairs distinctive, the outer pair forming a dense cockade like tuft

The antenna is characterized by possessing a large branched hair arising from a papilla on its side

The palmate hairs are borne upon the third to seventh abdominal segments The leaflets are highly characteristic being unlike those of the other genera (so far as described) lanceolate and serrated

The Characters of the Ovum — In *M barbirostris* and *M Sinensis* the ova belong to type 1 *i e*, the lateral floats not touching the marginal rim

In *M barbirostris* and *M Sinensis* there is a characteristic appearance of a well marked polygonal pattern upon the under surface

(A) Palpi *unbanded* — Last hind tarsus brown

1 *M barbirostris* one fringe spot India, Malaysia

2 *M Bancroftii* several fringe spots Australia

3 *M umbrosus* no fringe spot only one costal spot Malaysia

Last hind tarsus white

4 *M albotaeniatus* other hind tarsi much banded Malay

Last two hind tarsi white

5 *M Coustani* Madagascar

(B) Palpi *banded*—Last hind tarsus brown

6 *M Sinensis* wing fringe one pale spot China

Palpi banded last hind tarsus brown wing fringe unspotted Apex of palpi white

7 *M Vanus* costa two yellow spots wings distinctly spotted India Malay Philippines etc

8 *M Pseudopictus* wings without prominent spots Europe

9 *M minutus* wings two white costal spots Punjab

Apex of palpi black

10 *M nigerrimus* India

(C) Palpi banded last hind tarsi white

11 *M mauritianus* two hind tarsi white

12 *M Paludis* three hind tarsi white Africa

Note—D ... *Plumiger* and *Fenebrosus* are either *Barbirostris* or *Sinensis* according to THEOBALD Add *M Pseudo barbirostris* from the Philippine

Genus *Nyssorhynchus*—νυσσω to puncture bite ρυγχος proboscis

Mosquitoes mostly with legs spotted and banded or one or more tarsal segments pure white They are both domestic and wild mosquitoes They breed chiefly in pools with algae

and in holes. N. *Stephensi* will, however, breed in pots and tins. Larva: antennal hair simple. Ova, type 2.

Legs *unspotted*. Larva with outer pair of clypeal hairs markedly branched. Leaflets of palmate hairs with long filament.

1. N. *Fuliginosus*.—Probably = *Leucopus*, DÖNITZ. Costa four large and one or more small pale spots. Femora, pale band near the apex. Hind tarsi three and one fifth pure white. Palpi two narrow white bands, apex white (Fig. 53). India.

2. N. *Karwari*.—Legs not speckled, one and one fourth hind tarsal joints white. In fore and mid legs tarsal joints, except fourth and fifth, have apical white band. In hind legs tibia, first and second tarsus have apical bands; third and fourth have both apical and basal bands; the fifth is white. Palps *four white bands, two terminal* broad and equal, two basal narrow, apex white. India.

Legs markedly *spotted*. Larva with simple or slightly branched frontal hairs. *Filaments of palmate leaflets very short*.

3. N. *Stephensi*.—Syn. = A. *Metaboles*, THEOBALD. Tarsus without any segment of hind leg white. Legs brown, speckled with white; joints of fore and hind tarsi with apical spots. Wing costa, four broad prominent *black spots and two smaller* basal ones. The third largest spot has three typical spots beneath it on the first long vein. Fringe dark with pale areas. Palpi two broad apical white bands, one narrow basal; white scales between the last two bands. India.

4 *N Maculatus*—Resemble *N Stephensi* but is easily distinguished by tarsi Wings costa four large and two small basal spots Under the third largest spot are three black spots on the first long vein Legs with femora tibiae and metatarsi with broken creamy bands and spots Fore and mid tarsi with narrow yellow bands Hind tarsi with broad white ones *Last segment pure white* Palpi, four bands two unequal white apical bands then a small white one and a second towards the base

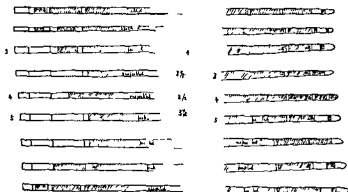


Fig 53

Hind Tarsi and Palpi N *Karwari* (6) N *Maculata* (1) N *Theobaldi* ( ) N *Fuliginosus* (3) N *Fuliginosus* (*Nagpurensis*) (4) N *Maculipalpis* (5) N *Jamesii* (7) N *Pretoriensis* (8) N *Willmori* ( )

5 *N Theobaldi*—Wings jet black with the costa interrupted by five white spots and an apical spot Legs brindled with white scales and a large sub apical white patch on the femora Two

and a quarter hind tarsi pure white then a black band then a small white one Palpi three white bands apex white, two apical bands equal a third narrow

A Nagpur variety which THEOBALD considers may be a distinct species has two and a half hind tarsi white and the tips of the palpi black India

6 A *Maculipalpis* = A *Jamesii* in *Reports to Royal Society* STEPHENS and CHRISTOPHERS Wing costa black with five white spots Legs black spotted with white last three hind tarsi pure white, and apex of next Palpi two broad white bands one apical a third narrow one towards the base The rest of the palpi spotted with white Length 5.5 mm India Africa

7 A *Jamesii* —Costa, four large and two small dark spots Legs brown fore femora and tibiae more or less *spotted* Hind legs, femora and tibiae with an apical white spot last three tarsi white and apex of next (Fig 53) The first tarsal segment of the fore leg has an indistinct median band Palpi black with white rings and white apical *joint closely related to A Fuliginosus*, but easily distinguished Length 3 to 3.5 mm *Cp* A *Maculipalpis* length 5.5 mm

A *Maculipalpis v Indiensis* —Hind legs not quite so banded as in the type Some variation in wing markings

8 A *Pretoriensis* —Clypeal hairs of larva simple Palps not mottled otherwise like A *Maculipalpis* The two white apical bands are further apart Second hind tarsus has a small black patch near its base Metatarsus mottled with white and black and has a broad white

apical band like the first tarsal The last two hind tarsi only white

9 *N Deceptor* (DONITZ) Sumatra *Deceptor* because very like *punctulatus* or *leucophyrus* Terminal half of *proboscis* white Terminal half of palpi white with a narrow black ring at the commencement of the third and fourth joint The ring around the light end of the second joint possessed by *A punctulatus* is wanting Legs similarly marked as in *leucophyrus* excepting the hind legs which have only a small light spot at the end of the tibia and not a broad white band

10 *N Willmori* (JAMES) Punjab Kashmir Wings four large and three small black areas Palpi three white bands the two terminal ones are equal and broad the third narrow and basal Legs dark brown thickly speckled with white spots The last tarsal segment of hind leg pure white

11 *N annulipes* Australia

12 *N Masteri* Australia

Genus *Cellia* —Wings densely scaled Palpi of ♀ densely scaled Easily recognized by the dense coating of irregular scales

Larvae (*C Pulcherrimus*)—Antennal hair simple Clypeal hairs outer pair branched Ova type 2

Last hind tarsi white

1 *C Pulcherrimus* $3\frac{3}{4}$ white Punjab
2 *C Bigotii* 3 Chili
3 *C Pharoensis* 1[$\frac{1}{3}$] Egypt Gambia
4 *C Argyrotarsis* $\frac{1}{2}$ Palpi three bands deep black basal band to last tarsus Acts as a host for *F nocturna* (VINCENT) West Indies

5 *C Albipes*, ½ Palpi, two bands West Indies, Brazil

Last hind tarsi yellow

6 *C Kochii*, 3 Malay

Last hind tarsus black

7 *C Squamosa* Africa

Genus *Aldrichia*—Wings much as in *Myzomyia*, for which genus it was originally mistaken

1 *Al Error*—Resembles *M Rossii* Easily distinguished by the abdominal scales India

Chapter XVII

THE HABITS OF ANOPHELES

GEOGRAPHICAL DISTRIBUTION

This is as yet far too imperfectly known for a close consideration of the subject to be of much value We may consider however that the *Anophelina* of some portions of Africa and some portions of India are known with a sufficient degree of exactitude to make a comparison of interest If however we take into consideration the distribution in the different parts of the same country the data are not nearly numerous enough Thus for instance *Pyretophorus Jeyporensis* has been recorded so far only from Madras (Jeypore) it is almost certain however that its distribution must be wider than this Again we have at present practically no data of that part of the world intervening between North Africa and India but it is of interest to note here that so far only two species have been found common to India and Africa viz Nyss *Maculipalpis* and M *Barbirostris* many genera are however common to Europe Africa and India and among these the species are sometimes closely allied Thus *Myzomyia funestus* (Africa) is closely allied though distinct from *Myzomyia Listoni* And further in the case of these two species we actually know from dissection and not from conjecture that they both

are found naturally conveying malarial sporozoits, and both are associated with areas of high endemicity The following is a complete list of the known *Anophelina* Where the same species occurs in more than one country we have indicated the fact by inserting in brackets the number under which it first occurred in the list —

Europe

1 A Maculipennis
2 A Bifurcatus
3 A Nigripes
4 Pyr Superpictus
5 Mym Hispaniola (and Teneriffe)
6 Myzo Pseudopictus

Palestine

7 Pyr Palestinensis
M Pseudopictus
[1] A Maculipennis
[4] P Superpictus

North America

8 A Maculipennis (? European species)
[3] A Nigripes
A *Pseudo punctipennis*
[2] A bifurcatus
9 A Punctipennis
10 A Crucians

South America and West Indies

11 C Argyrotarsis
12 C Bigotii
13 Mym Lutzii
14 Arri *Maculipes*
15 Cy *Grabhamii*
16 Cyclo Medio punctatus
17 Steth Nimba
18 C Albipes

Africa

19 Mym funesta
20 Myzo Paludis
21 Mym Rhodesiensis
22 C Squamosu
[4] Pyret Superpictu (West Coast and Mashonaland)
23 Pyret Costalis
24 C Pharoensis (Gambia Egypt)
25 Mym Longipalpis
26 Pyret Marshallii (Mashonaland)
M Coustani (Madagascar)
27 Pyret Cinereus (S Africa)
28 Mym Mauritianus
29 Mym Hebes (E A and S W A)
30 Pyret Merus
31 Nys Maculipalpis
32 Nys Pretoriensis
33 Myzo Barbirostris
34 A Algeriensis
35 Mym *Impunctus (Egypt)*
36 Pyret Chaudoyei (Algeria)

India

37	Myzo *Nigerrimus*	48	Mym *Culicifacies*
[3]	Myzo Barbirostris	49	Mym Turkhudi
[31]	Nysso Maculipalpis	50	Mym Elegans
38	Nysso Jamesii	51	Myzo minutu
39	Nysso Stephensi	5	Myzo varus
40	Nysso Theobaldi	53	Ce Fulcherrima (Punjab)
41	Nysso Maculatus	54	A Lindesayii (hill species)
4	*Nysso Willmori*		
43	Nysso Karwari	55	A Immaculatus (very rare)
44	Nysso fuliginosus		
45	Mym Rossii (everywhere)	56	A Aitkenii (Goa)
46	Mym Listoni	57	A Gigas (hill specie)
47	Mym Leptomeres	58	Ald error
	M Indiensis		P Jeyporen i

Malaysia

59	Mym Aconita		P Minimus (China)
60	Mym Ludlowii	65	Nysso deceptor
61	Mym Albirostris	66	Mym umbrosus
6	Mym Punctulatus	67	Mym albotaeniatus
63	Mym Kochii	[33]	Myzo plumiger or [64] (Donitz)
[45]	Mym Rossii	[8]	Mym tenebrosus (Donitz)
[46]	Mym Listoni	[57]	Mym Leucosphyrus (Donitz)
[5]	Mym varus		
[51]	Mym minutus		
[34]	Myzo barbirostris	[44]	Nyss Leucopus (Donitz)
64	*Myzo sinensis (China)*	[3]	*Pyret gracilis (Donitz)*
[41]	Nysso maculatus	[45]	Mym vagus (Donitz)
	M 1e selatum		

Australia

68	Myzorhynchus Bancroftii	71	N Ma t n
69	*A Stigmaticu*	7	*P Atratipe*
70	N Annulipe		

NOTE —From the Philippine we have M Pseudo barbirostris and A Philippinensis and three unclassified—A Vincenti (Tonkin) A Farauti and A Pursati making a total of over eighty

P

Seasonal Prevalence.—Few observations have been made on this point. It would appear that there is one simple explanation which, at least in part, will account for the prevalence of a particular species at a particular time, and its appearance or disappearance at others. We have already shewn how selective the *Anophelina* are in their choice of breeding grounds, consequently if at any time, *e.g.*, the dry season, a suitable breeding ground does not exist a particular species or genus of the *Anophelina* may be absent.

Thus we found in Nagpur (India, C.P.), during the dry season in those places where shallow puddles had dried up *Myz. Rossii* was rare but it abounded wherever puddles still remained. Where weedy lakes existed *Nyss. fuliginosus* was common, elsewhere rare. Now these conditions are directly dependent on the rainy season, and where vast areas of weedy swamp are formed during the rains then *M. Nigerrimus* prevailed to disappear when the swamps dried up. In temperate climes, the temperature is, no doubt, an important factor, the onset of the cold weather causing a general hibernation. We may quote the following observations:—

Nyssorhynchus Pretoriensis.—This species first appeared February 10 and gradually became more prevalent, superseding the other common species *(Pyret. Cinereus)* in April (THEOBALD, *Monograph of the Anophelina*, p. 99).

Pyretophorus Chaudoyei.—'In the winter up to June one only sees *C. Pipiens.* These then disappear and *Pyret. Chaudoyei* appears' (THEOBALD, p. 70).

The incidence of *Pyret. Chaudoyei* is said to be

accompanied by the recrudescence of severe malaria but it ought also to be definitely shewn that sporozoits are present in this species and presumably that the sporozoit rate increases with the outbreak of malaria

The Hibernation of Anopheles

1 *Hibernation of the Adult Insects* Annett and Dutton describe the finding of *A maculipennis* during the winter in England in cellars lumber rooms and other cold places but not in stables where the temperature is higher

They observed the following points —

(i) The attitude is peculiar the insect lying quite flat upon the surface with its legs spread out In this position the under surface of the thorax touches or nearly touches the wall

(ii) Only females are found and these are always fertilized and have the spermatheca filled with spermatozoa

(iii) The insects are difficult to arouse and very sluggish in any movements they make

(iv) They do not feed unless the temperature is raised If kept at a low temperature (provided the air is moist) they remain for weeks without feeding

(v) If roused by raising the temperature they feed readily and the ovaries rapidly develop Eggs are laid and in most cases the female dies after their deposition

Culex, likewise behave in much the same way

2 *Hibernation of the Larva* —The larvae of certain *Anopheles* appear able to resist low temperatures and are found even when parts of the water

Seasonal Prevalence.—Few observations have been made on this point. It would appear that there is one simple explanation which, at least *in part, will account for the prevalence of a* particular species at a particular time, and its appearance or disappearance at others. We have already shewn how selective the *Anophelina* are in their choice of breeding grounds, consequently, if at any time *e.g.* the dry season a suitable breeding ground does not exist, a particular species or genus of the *Anophelina* may be absent.

Thus we found in Nagpur (India C.P.) during the dry season, in those places where shallow puddles had dried up, *Myz. Rossii* was rare but it abounded *wherever puddles still remained.* Where weedy lakes existed, *Nyss. fuliginosus* was common elsewhere rare. Now these conditions are directly dependent on the rainy season, and where vast areas of weedy swamp are formed during the rains, then *M. Nigerrimus* prevailed, to disappear when the swamps dried up. In temperate climes, the temperature is no doubt an important factor the onset of the cold weather causing a general hibernation. We may quote the following observations:—

Nyssorhynchus Pretoriensis.—This species first appeared February 10 and gradually became more prevalent superseding the other common species *(Pyret. Cinereus)* in April (THEOBALD *Monograph of the Anophelina* p. 99).

Pyretophorus Chaudoyei.—"In the winter up to June one only sees *C. Pipiens.* These then disappear and *Pyret. Chaudoyei* appears" (THEOBALD p. 70).

The incidence of *Pyret. Chaudoyei* is said to be

accompanied by the recrudescence of severe malaria but it ought also to be definitely shewn that sporozoits are present in this species and presumably that the sporozoit rate increases with the outbreak of malaria

THE HIBERNATION OF ANOPHELES

1 *Hibernation of the Adult Insects* ANNETT and DUTTON describe the finding of *A maculipennis* during the winter in England in cellar lumber rooms and other cold places but not in stables where the temperature is higher

They observed the following points —

(i) The attitude is peculiar the insect lying quite flat upon the surface with its legs spread out In this position the under surface of the thorax touches or nearly touches the wall

(ii) Only females are found and these are always fertilized and have the spermatheca filled with spermatozoa

(iii) The insects are difficult to arouse and very sluggish in any movements they make

(iv) They do not feed unless the temperature is raised If kept at a low temperature (provided the air is moist) they remain for weeks without feeding

(v) If roused by raising the temperature they feed readily and the ovaries rapidly develop Eggs are laid and in most cases the female dies after their deposition

Culex likewise behave in much the same way

2 *Hibernation of the Larva* —The larvae of certain *Anopheles* appear able to resist low temperatures and are found even when parts of the water

Seasonal Prevalence.—Few observations have been made on this point. It would appear that there is one simple explanation which, at least in part, will account for the prevalence of a particular species at a particular time, and its appearance or disappearance at others. We have already shewn how selective the *Anophelina* are in their choice of breeding grounds, consequently if at any time, *e.g.*, the dry season, a suitable breeding ground does not exist a particular species or genus of the *Anophelina* may be absent.

Thus we found in Nagpur (India, C.P.), during the dry season in those places where shallow puddles had dried up *Myzomyia Rossii* was rare but it abounded wherever puddles still remained. Where weedy lakes existed *Nyss. fuliginosus* was common, elsewhere rare. Now these conditions are directly dependent on the rainy season, and where vast areas of weedy swamp are formed during the rains, then *M. Nigerrimus* prevailed to disappear when the swamps dried up. In temperate climes, the temperature is no doubt, an important factor: the onset of the cold weather causing a general hibernation. We may quote the following observations:—

Nyssorhynchus Pretoriensis.—This species first appeared February 10 and gradually became more prevalent superseding the other common species *(Pyret. Cinereus)* in April (THEOBALD, *Monograph of the Anophelina*, p. 99).

Pyretophorus Chaudoyei.—"In the winter up to June one only sees *C. Pipiens*. These then disappear and *Pyret. Chaudoyei* appears (THEOBALD, p. 70).

The incidence of *Pyret. Chaudoyei* is said to be

accompanied by the recrudescence of severe malaria but it ought also to be definitely shewn that sporozoits are present in this species and presumably that the sporozoit rate increases with the outbreak of malaria

The Hibernation of Anopheles

1 *Hibernation of the Adult Insects* Annett and Dutton describe the finding of *A maculipennis* during the winter in England in cellars lumber rooms and other cold places but not in stables where the temperature is higher

They observed the following points —

(i) The attitude is peculiar the insect lying quite flat upon the surface with its legs spread out In this position the under surface of the thorax touches or nearly touches the wall

(ii) Only females are found and these are always fertilized and have the spermatheca filled with spermatozoa

(iii) The insects are difficult to arouse and very sluggish in any movements they make

(iv) They do not feed unless the temperature is raised If kept at a low temperature (provided the air is moist) they remain for weeks without feeding

(v) If roused by raising the temperature they feed readily and the ovaries rapidly develop Eggs are laid and in most cases the female dies after their deposition

Culex likewise behave in much the same way

2 *Hibernation of the Larva* —The larvae of certain *Anopheles* appear able to resist low temperatures and are found even when parts of the water

Seasonal Prevalence—Few observations have been made on this point It would appear that there is one simple explanation which at least in part will account for the prevalence of a particular species at a particular time, and its appearance or disappearance at others We have already shewn how selective the *Anophelina* are in their choice of breeding grounds, consequently, if at any time *e g*, the dry season a suitable breeding ground does not exist a particular species or genus of the *Anophelina* may be absent

Thus we found in Nagpur (India, C P), during the dry season, in those places where shallow puddles had dried up, *Myz Rossii* was rare but it abounded wherever puddles still remained Where weedy lakes existed, *Nyss fuliginosus* was common elsewhere rare *Now these conditions are directly* dependent on the rainy season and where vast areas of weedy swamp are formed during the rains, then *M Nigerrimus* prevailed to disappear when the swamps dried up In temperate climes, the temperature is no doubt, an important factor the onset of the cold weather causing a general hibernation We may quote the following observations —

Nyssorhynchus Pretoriensis—This species first appeared February 10 and gradually became more prevalent superseding the other common species *(Pyret Cinereus)* in April (THEOBALD *Monograph of the Anophelina* p 99)

Pyretophorus Chaudoyei—'In the winter up to June one only sees *C Pipiens* These then disappear and *Pyret Chaudoyei* appears (THEOBALD p 70)

The incidence of *Pyret Chaudoyei* is said to be

accompanied by the recrudescence of severe malaria but it ought also to be definitely shewn that sporozoits are present in this species and presumably that the sporozoit rate increases with the outbreak of malaria

The Hibernation of Anopheles

1 *Hibernation of the Adult Insects* ANNETT and DUTTON describe the finding of *A maculipennis* during the winter in England in cellars lumber rooms and other cold places but not in stables where the temperature is higher

They observed the following points —

(i) The attitude is peculiar the insect lying quite flat upon the surface with its legs spread out In this position the under surface of the thorax touches or nearly touches the wall

(ii) Only females are found and these are always fertilized and have the spermatheca filled with spermatozoa

(iii) The insects are difficult to arouse and very sluggish in any movements they make

(iv) They do not feed unless the temperature is raised If kept at a low temperature (provided the air is moist) they remain for weeks without feeding

(v) If roused by raising the temperature they feed readily and the ovaries rapidly develop Eggs are laid and in most cases the female dies after their deposition

Culex likewise behave in much the same way

2 *Hibernation of the Larva* —The larvae of certain *Anopheles* appear able to resist low temperatures and are found even when parts of the water

Seasonal Prevalence—Few observations have been made on this point It would appear that there is one simple explanation which at least in part will account for the prevalence of a particular species at a particular time and its appearance or disappearance at others We have already shewn how selective the *Anophelina* are in their choice of breeding grounds, consequently, if at any time, *e g* the dry season, a suitable breeding ground does not exist, a particular species or genus of the *Anophelina* may be absent

Thus we found in Nagpur (India C P), during the dry season, in those places where shallow puddles had dried up, *Myz Rossii* was rare, but it abounded wherever puddles still remained Where weedy lakes existed, *Nyss fuliginosus* was common elsewhere rare Now these conditions are directly dependent on the rainy season, and where vast areas of weedy swamp are formed during the rains, then *M Nigerrimus* prevailed to disappear when the swamps dried up In temperate climes the temperature is no doubt, an important factor the onset of the cold weather causing a general hibernation We may quote the following observations —

Nyssorhynchus Pretoriensis—This species first appeared February 10 and gradually became more prevalent superseding the other common species *(Pyret Cinereus)* in April (THEOBALD *Monograph of the Anophelina* p 99)

Pyretophorus Chaudoyei—' In the winter up to June one only sees *C Pipiens* These then disappear and *Pyret Chaudoyei* appears (THEOBALD p 70)

The incidence of *Pyret Chaudoyei* is said to be

accompanied by the recrudescence of severe malaria but it ought also to be definitely shewn that sporozoits are present in this species and presumably that the sporozoit rate increases with the outbreak of malaria

The Hibernation of Anopheles

1 *Hibernation of the Adult Insects* ANNETT and DUTTON describe the finding of *A maculipennis* during the winter in England in cellars lumber rooms and other cold places but not in stables where the temperature is higher

They observed the following points —

(i) The attitude is peculiar the insect lying quite flat upon the surface with its legs spread out In this position the under surface of the thorax touches or nearly touches the wall

(ii) Only females are found and these are always fertilized and have the spermatheca filled with spermatozoa

(iii) The insects are difficult to arouse and very sluggish in any movements they make

(iv) They do not feed unless the temperature is raised If kept at a low temperature (provided the air is moist) they remain for weeks without feeding

(v) If roused by raising the temperature they feed readily and the ovaries rapidly develop Eggs are laid and in most cases the female dies after their deposition

Culex likewise behave in much the same way

2 *Hibernation of the Larvae* — The larvae of certain *Anopheles* appear able to resist low temperatures and are found even when parts of the water

are frozen over Under these circumstances they grow extremely slowly, if at all

3 *Hibernation of Eggs*—There is a certain amount of evidence to shew that eggs can survive for some months in moist earth exposed to frost, etc For young larvae have been found in fresh pools in the winter under conditions that made it unlikely that the eggs had been deposited there on the appearance of water The resistance of eggs to drying under a tropical sun is, however, practically nil In the case of the eggs of *Culex fatigans* Miss Evans made the following observation in Calcutta — Desiccation of the egg boat for one day was generally sufficient to prevent the larvae from hatching out though one or two larvae did hatch out of an egg boat which had been dried (? exposed to the sun) for six days

Mode of Dispersal of Anophelina

There is no evidence existing at present to show that mosquitoes habitually disperse any considerable distance from their breeding grounds In fact the evidence is completely against such a dispersal, and broadly speaking, the *Anophelina* remain where they were developed and in the native huts where they find abundant food

That various accidental modes of distribution occur is equally certain *e g* —

1 On trains, boats and even ocean going steamers they may be carried long distances *e g* from West Africa and South America to England but it remains to be shewn that *Anophelina* thus introduced ever effect a permanent habitation, even when the removal by this means is from one portion of the tropics to another

2 Locally streams and canals may carry larvae and ova long distances perhaps miles

3 *Winds* —The maximum distance that the *Anophelina* can be carried in this way is quite uncertain Nearly all of the excessive distances that have been given as possible flights refer to *Culex* It appears certain moreover that the *Anophelina* dislike wind and seek shelter from it

4 *Trees Plantations Bush Jungle* —These elements undoubtedly hinder the flight of *Anopheles* and on the contrary open spaces promote their diffusion It is necessary to bear this fact in mind where a belt of jungle screens off a source of *Anopheles* (larvae) which may find an opportunity of becoming infected later

DOMESTIC AND WILD SPECIES OF ANOPHELES

Anopheles are mostly found in association with native dwellings where there is abundance of food (blood) *Anopheles* are also generally abundant where cattle are kept

Certain species are distinctly domestic in their habits e g *Myz Rossii Pyr costalis* Nyss *Stephensi* and others They are found resting in the daytime in the thatch of huts and they breed close at hand in the nearest puddle They may, however fly up to half a mile if there are no breeding places closer

Other species are not peculiar to houses but are also found breeding in streams and pools in the jungle far from habitations Such species are *Nyss Maculata* Nyss *Theobaldi*

The mosquitoes of the genus *Myzorhynchus* on the contrary are wild *Anopheles* They are

only occasionally found in houses They breed in extensive bodies of water swamps rivers jungle pools etc It is *Anopheles* of this type which chiefly frequent one s tent when this is pitched in remote and especially in swampy jungle The more common species of these wild *Anopheles* are *M barbirostris M Sinensis, M paludis*

Nature of Food

The normal food of the female (domestic) *Anopheles* is blood In nature they appear to feed every night the stomach never becoming empty In *Anopheles* caught under natural conditions the stomach contents generally shew blood in two or three stages of digestion

Female *Anopheles* readily drink water, especially if they have been kept for some time in a dry bottle It seems doubtful whether vegetable juices form an important article of food as appears to be the case with some of the *Culicidae* Male *Anopheles* can be seen feeding upon banana and other fruit juices but are notwithstanding, found dead about the second or third day of captivity

Under some conditions the females do not feed upon blood *e g A maculipennis* in England (Theobald)

Bancroft states that *Nyss Annulipes* will live for a month on dates, but only for three days on bananas

Time of Feeding

The usual time for feeding of *Anopheles* is after dark, more especially in the early night and before dawn Occasionally some *Anopheles* may

be found biting in broad daylight and ANNETT and DUTTON state that *Anopheles* feed readily in certain parts of Nigeria by day. Possibly certain species feed more readily by day than others.

We have ourselves seen on rare occasions *M. Rossii* attempting to feed in the daytime, and GRAY (B.C.A.) says that *Ce. albipes* when disturbed will bite at any time of the day or night.

On the whole, however, the *Anophelina* are strictly nocturnal in their habits. Nor do they hover around lamps as has been supposed. Of *A. bifurcatus* BLANCHARD states that it bites fiercely at dusk, but at night practically not at all. At dawn, however, it begins again, and it bites at all times in shady places, outhouses, etc.

DISTANCE OF FLIGHT

The *maximum* distance that *Anopheles* can fly requires further study. In questions of flight the species of mosquito should always be noted. Observations upon the flight of mosquitoes have so far been vague and uncritical. With regard to *Anopheles* on ships it must be borne in mind that they have not necessarily come from the land on the night upon which they appear, but may have come on board when the ship was in port, or even have been bred on board. In certain villages in India studied by us, *Myz. culicifacies*, *Nyss. Stephensi* and *Nyss. fuliginosus* were always present in abundance if there were extensive breeding grounds within quarter of a mile. Where villages were distant half a mile from extensive breeding grounds they contained few or no *Anopheles*. The only exceptions to this rule were when breeding places had only recently dried up. In the case of

the above species they undoubtedly fly fairly readily quarter of a mile but half a mile appears to be beyond the normal distance of flight

RELATION TO COLOUR, ODOUR OR OBJECTS ETC

Anyone who in the tropics, has left his wardrobe open at sunrise and then closed it and again examined it some time later will have often observed the well known fact that, on his white clothes, few or no mosquitoes are resting but that on his blue serge clothes there may be dozens He will have noted too that outside his mosquito net it is on the shady side that the mosquitoes remain longest until from here also, they fly away as the fierce sun rises

He will have noted, too that *Anopheles* as well as *Culex* have a predilection for certain smells Old boots and blacking attract them strongly and the leather of a saddle room is their favourite haunt *Anopheles*, too much prefer the odoriferous skin of the native to that of the European as experiments made by us in Sierra Leone clearly shewed

NUTTALL and SHIPLEY have made some laboratory experiments *on the influence of colour*, and find that navy blue is the colour most preferred by *A maculipennis* and yellow the one most shunned

As, however the *Anophelina* at least are nocturnal in their habits and prefer biting unclothed portions of the body, the colour of one's clothing will not be much protection If white or yellow socks can prevent the persistent attacks of *Stegomyia* it would indeed be a practical boon

To various trees and plants has been ascribed a repellent effect upon mosquitoes None of these statements has so far borne a critical examination

LENGTH OF LIFE OF MOSQUITOES

The length of life of mosquitoes under suitable conditions is probably considerable several weeks to months In captivity they may if suitably housed and constantly fed be kept alive for days weeks and even months A mosquito kept some time in captivity becomes infirm and readily falls into the water whilst laying its eggs It also finds difficulty in hanging on to smooth glass and even though a rough surface is supplied the insect is constantly found on the bottom of the cage resting in a horizontal position After laying eggs such infirm mosquitoes generally die the same night In nature *Anopheles* certainly remain alive in huts for one or two months and possibly longer After the drying up of all breeding places the winged *Anopheles* do not much diminish in number for several weeks If the drying up continues the numbers gradually diminish but specimens may be caught up to two months or more afterwards

AESTIVATION OF MOSQUITOES

In very hot and dry countries the *Anopheles* which remain through the dry season appear to exhibit some peculiarities in their habits —

1 Unlike hibernating mosquitoes they feed regularly and are found full of blood

2 The ovaries are in the majority large and the ova fully developed

Chapter XVIII

ANOPHELES—THE OVUM

The Ovum

Anopheles in captivity generally lay their eggs on some floating object but also upon the surface of the water When laid on a solid object, and even when laid on the water, the eggs are deposited in a piled up mass Later, the ova if on water, often form very regular and beautiful patterns Brick red masses of eggs are sometimes laid These do not develop further

Observe (i) the arrangement in equilateral triangles and star patterns (Fig 19)

(ii) The arrangement in rows of eggs lying side by side

Both patterns are dependent upon the shape of the individual ovum ova belonging to type 1 forming stars and ova belonging to type 2 rows

The number of ova varies but is usually about one hundred The size of the ovum varies with different species from about 0 6 to 1 0 mm

Examination of Anopheles Ova

Anopheles ova (with one exception as yet described) are boat shaped with an approximately

flat upper surface and a deeply convex lower surface. One end which contains the head of the embryo is blunter and broader than the other. During the act of hatching this end is forced open by the escaping larvae.

1. *The Upper Surface*.—Observe that the upper surface is generally granular or tuberculated in appearance. At either extremity it is continuous with the pointed ends of the ovum and in this position there are usually several small polygonal areas. The width of the upper surface and the extent to which it is encroached upon by the floats varies in different species.

2. *The Lower Surface*.—The lower surface is generally smooth and dark grey. In damaged ova a silvery membrane will be seen partly detached shewing a deep shiny black surface beneath. The silvery membrane is the outer covering of the egg and formed by the layer of follicular epithelium (Fig. 39). In some species the lower surface is marked with silvery lines forming a reticular pattern.

3. *The Floats*.—Occupying about the middle third of the side of the ovum is a remarkable structure—the float. This consists of a very delicate membrane continuous with the chitinous cuticle covering the whole ovum and containing air cells.

The floats are generally oval in shape and shew regular transverse corrugations. The shape and position of the floats vary considerably in the different species.

4. *The Frill*. Around the margin of the upper surface (forming the gunwale of the boat) there is in some species a gleaming white frill like

structure This is striated in appearance, but portions of it may (in some species) be free from striations In other species the appearance is rather that of a white striated rim In all species of *Anopheles* ova yet described, a striated *frill or rim is present* The width and extent of the frill vary in different species

Three very distinct types of ovum have been seen by us—

Type 1 —Ova have the upper surface very narrow with the lateral floats not touching the margin (Fig 54 1)

The species with ova of this type are—

M barbirostris	*M culicifacies*
M Sinensis	*M Listoni*

sub sp *nigerrimus*

Type 2 —Ova having a more or less broad upper surface with the lateral floats touching the margin (Fig 54 2 3 4, and 6)

Species having ova of this type are—

M Rossii	*N fuliginosus*
Ce pulcherrimus	*N Stephensi*

Type 3 —Ova with no floats and with upper surface rudimentary (Fig 54 5)

One species only as yet described has ova of this type viz —

M Turkhudi

Species having ova of the first type have in all cases been species breeding in either open natural waters or running streams

Species with ova of the second type are in general found breeding in pools

The only ova as yet systematically described are those of the Indian *Anopheles* Further observations will probably add further types to the above

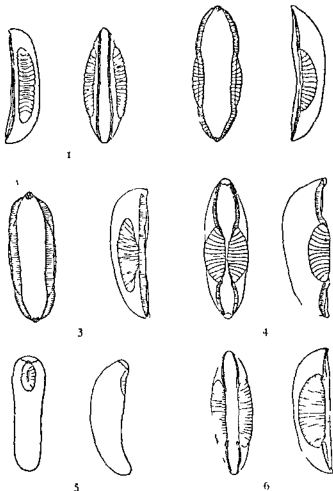


Fig 54 Ova of Anophelina
1 M *Culicifaci* C *Fulcherrimu* 3 M *Rossii*
4 N *Stephensi* 5 M *Turkhudi* 6 N *Maculipalpis*

Within each type great variation usually exists in the different species The following are the most notable variations found —

1 *The Frill* —The width The continuity of the frill around the whole of the margin of the upper surface or its replacement in the middle third by the floats The extent of striation of the frill The presence of a striated rim only

2 *The Floats* —The position, placed forwards and encroaching on the upper surface or laterally situated The shape oval, globular, or scallop shell

3 *The Lower Surface* —Whether ornamented or not with silvery reticulated pattern

The following is a brief résumé of the characters of the ova of *Anopheles*, as far as these have been described —

Type 1 *M Sinensis* sub sp *nigerrimus*

Ovum —Upper surface very narrow Floats do not touch margin of upper surface Lower surface of ovum ornamented with polygonal markings

M barbirostris

Ovum —Upper surface very narrow Floats do not touch margin of upper surface Lower surface of ovum ornamented with polygonal markings

M culicifacies

Ovum —Upper surface very narrow Floats do not touch margin of upper surface Lower surface not ornamented A short but distinct fringe is continued around margin of upper surface

M. Listoni

Ovum.—Upper surface very narrow. Floats do not touch margin of upper surface. Lower surface not ornamented. A small fringe passes around margin of upper surface.

Type 2. *M. Rossii*

Ovum.—Upper surface broad. Fringe very well developed and striated throughout whole length. Floats scallop-shell shape and touch margin of interior surface. Lower surface not ornamented.

Ce. pulcherrimus

Ovum. Upper surface broad. Floats touch margin of upper surface. Fringe well developed around margin of upper surface. Striations are not present in that portion of the fringe lying over the floats. Lower surface not ornamented.

N. fuliginosa

Ovum. Upper surface moderately broad. Floats touch margin of upper surface. Floats long and narrow. Fringe around upper surface only indicated by white border. Lower surface not ornamented.

N. Maculipalpis

Ovum. The upper surface is rather narrow. The floats are rather short and oval, and are placed far forwards as in the ovum of *N. Stephensi*, though less markedly so. The fringe is fairly developed but is not continued over the floats.

N. Stephensi.

Ovum.—Upper surface broad except in central portion where encroached upon by floats. Floats placed on margin of upper surface so that they touch, or nearly touch one another in middle line. Floats short and almost globular. Fringe not well developed. Lower surface not ornamented.

N. Theobaldi.

Ovum.—As the females of this species have only been very occasionally caught by us in houses we have not been able to describe the ovum as deposited by the insect. Fully developed ova removed from a bred specimen showed, however, that the ovum resembled that of *N. Maculipalpis.* The floats were rather short and situated far forwards as in *N. Stephensi.* The fringe is fairly developed but does not pass over the floats.

Type 3. *A. Turkhudi.*

A. Turkhudi is a very aberrant type so far as the ovum and larva are concerned. Both the ovum and larva approach to the characters of the *Culex* ovum and larva. *The eggs were laid upon* a floating object. When placed upon water they sink. They were laid in the heaped up manner sometimes adopted by *Anopheles* especially *M. Rossii* and *N. maculipalpis.* The chief characters of the ovum are:—

1. No separation of an upper surface as in all other *Anopheles* ova. At the thicker end of the ovum there is an oval area about a quarter the length of the whole egg. This is glistening

white and striated and probably represents the upper surface of other *Anopheles* ova

2 There are no floats or any markings representing them

3 There is a pale area at the thicker end of the egg with a scalloped edge

4 The ovum is otherwise without markings

It is obvious that the characters of the ovum are of considerable importance in the classification of *Anopheles* and every care should be taken to describe these in as great detail as possible

In making drawings of the ova of *Anopheles* it is convenient to use an eyepiece micrometer the width of the frill and of the upper surface and the length of the floats as compared with the length of the ovum may all be readily and accurately noted

To Mount Ova

No thoroughly satisfactory method is known to us but although imperfect any of the following methods will give specimens in which some at least of the ova preserve most of their characteristics

1 Place the eggs on a slide which has been made slightly sticky with balsam and then mount them in a drop of balsam and place a cover glass over them

2 Mount in two per cent formalin solution and ring the coverglass with balsam or shellac

3 Mount in glycerine and ring the specimen

4 Mount in a drop of cedar wood oil

3 *The Eyes* The eyes are situated laterally, and can be seen both from the dorsal and ventral surface Their size and appearance vary with the age of the larva In the full grown larva a crescentic compound eye is seen on either side, and behind this a single pigment mass (simple eye) The compound eye is absent in the first stages and becomes more prominent as the larva approaches maturity

4 *The Mouth Parts*

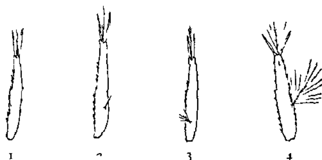


Fig 55 Lateral Hairs of Antennae
1 *M Rossii* 2 *N Stephensi* 3 *N I and savii* 4 *M Nigerrimus*

(*a*) Two very conspicuous bodies resembling somewhat shaving brushes are protruded or withdrawn under the overhanging clypeus These are the feeding brushes, and are employed in collecting the minute food particles on which the larva feeds

(*b*) On either side of the mouth is a broad blade like structure carrying several leaflets and some hairs (maxillary palps)

(c) Below the feeding brushes and not so easily visible are two stout bodies with comb like projections (mandibles)

(d) In the middle inferior line lies a conical toothed structure The under lip of Meinert (Fig 23)

(e) In the fully grown larva a snout like process covered with short hairs projects forwards in the middle line between the brushes

The front portion of the head projects between the antennae as a semi circular smooth area In front of this is a protrusion overhanging the base of the brushes (the clypeus)

5 *The Clypeal Hairs* —These are four or six in number Two spring from the extreme front of the clypeus near the middle line two from the outer corner of the clypeus immediately over the feeding brushes and two usually very small and not always present behind the origin of the others

The clypeal hairs are best seen when the feeding brushes are retracted They must not be confounded with certain other hairs on the larval head These are

(i) Six large branched hairs arising from the prominence lying between the bases of the antennae

(ii) Four similar branched hairs but smaller situated further back (NUTTALL and SHIPLEY)

The hairs exhibit great variation in different species but are quite constant in the one species A minute description of these hairs is of great importance in describing the specific characters of the larva

Clypeal Hairs of Larvae —

(i) The four anterior hairs may be quite simple and unbranched *Anopheles* having larvae of this type are *M Rossii*, *N Stephensi* *M culicifacies* *M Listoni* *M Turkhudi* (all Indian species)

(ii) All four anterior hairs may shew small lateral branches *P Jeyporensis*

In *A maculipennis* all four hairs are branched, the outer pair form distinct tufts

(iii) The outer pair may be markedly branched (from six to twelve hairs or more arising from near the point of origin) *e g*, *Ce pulcherrimus*

(iv) The outer pair may be developed into a close tuft (cockade) *e g*, *M barbirostris*

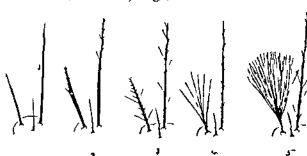


Fig 56 Clypeal Hairs of Larvae

1 *M Rossii* *N Stephensi* *M Culicifacies* *M Listoni* 2 *N Maculipalpis* 3 *P Jeyporensis* 4 *Ce Pulcherrimus* 5 *M Sinensis* *M Barbirostris*

The two hairs situated behind these may instead of being very short and inconspicuous be long and prominent *e g* *M Turkhudi*

6 *The Thorax* —The thorax in the adult larva is large and globular In the young larva it is not so broad as the head but becomes proportionately larger as the larva advances in age

Numerous hairs arise from the front and sides of the thorax. None of these vary perceptibly in different species. A number of large hairs arising from papillae on the lower surface are capable of being used almost as a means of progression when the larva is in very shallow water.

(*a*) Observe on the dorsum of the thorax a short but extremely stout and strong hair unlike the others projecting outwards and forwards.

(*b*) A flap-like body may with careful focussing be seen lying at the base of the most anterior hairs on either side.

(*c*) In some species of *Anopheles* a single pair of palmate hairs similar to those on the abdominal segments are found upon the thorax. In others they are rudimentary or absent. The presence of well developed palmate hairs on the thorax is of specific importance.

(i) It is well developed and functionally active in *M. culicifacies*, *M. Listoni*, *P. Jeyporensis*.

(ii) It is rudimentary or absent in all other larvae as yet described.

7. *The Abdomen.*—The first seven segments are very similar in shape. The eighth carries the opening of the air tube and the ninth some curious papillae and large hairs.

Each of the first two segments carries on each side a pair of long feathered hairs. The third carries a single similar hair. On the other segments there are much smaller and unfeathered hairs. On all the segments there are groups of small hairs which do not appear to vary and which are not of sufficient importance to describe here. None of the above structures appear to vary in different species.

Skeleton Larvae

Examination of Palmate Hairs—The most important appendages of the abdominal segments are certain small fan or palm leaf shaped hairs attached by a short stalk to the outer dorsal portions of certain of the segments (Fig 57A) The number of segments bearing well developed palmate hairs varies in different species

Place the larva under a coverglass in a drop of water or use a permanently mounted larva

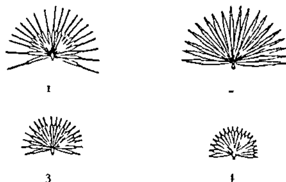


Fig 57A Palmate Hairs of Larvae
1 *M Rossii* 2 *M Vigerrimus* 3 *M Listoni*
4 *N Maculata*

1 Determine the number of segments which carry distinct and large palmate hairs and those carrying ill developed ones

2 Carefully cut out with a needle two or three central abdominal segments Crush these

The following arrangement of these hairs is found in Indian species of larvae the only larvae as yet systematically described

1 Fully developed hairs on all segments (one to seven) and on the thorax

P Jeyporensis
M Listoni
M culicifacies

2 Fully developed hairs on the second to seventh or third to seventh segments Rudimentary hairs on the second or even first abdominal segments and on the thorax

N Stephensi
N maculata
N Theobaldi

3 Palmate hairs confined to the third fourth fifth sixth and seventh segments

M Sinensis
M barbirostris
A maculipennis (NUTTALL and SHIPLEY)

4 Palmate hairs confined to the fourth fifth and sixth segments

M Turkhudi

The Leaflets — In the well grown larvae each palmate hair consists as a rule of nineteen or twenty leaflets arising close together from a short stalk and forming a semi circular fan When collapsed as is the case when the larvae is beneath the surface these hairs are inconspicuous When however the larvae takes up its characteristic attitude at the surface of the water these spread out fan like and are very striking objects under the microscope In the freshly hatched larva the separate leaflets appear to be folded together so that the hair has the appearance of a single lanceolate structure About the third day the hairs are seen with seven to eight uniformly lanceolate leaves Very soon after this they take on

the characters seen in the hairs of the mature larva

In the mature larva the leaflets shew much variation in the different species In most species, the leaflets terminate rather suddenly in a number of jagged points or notches whilst the central portion continues as a more or less fine filament

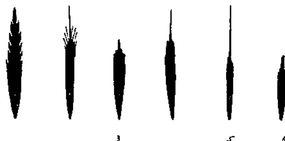


Fig 57B *Leaflets of Palmate Hairs*
1 *M Sinensis M Barbirostris* N *Lindesayii*
3 *N Theobaldi N Stephensi* 4 *M Listoni M Culicifacies*
5 *M Rossii* 6 *M Turkhudi*

The character of the notching and the relative length of the filament to the leaflet are of specific importance The following types of leaflets are known —

1 The leaflets are unbrokenly lanceolate in shape with saw like notches along the edge of the outer half There is no distinct terminal filament

M Sinensis
M barbirostris

2 The filament is long and filamentous

M Rossii
M culicifacies
M Listoni
N fuliginosus

Further differences are seen in the case of most of the above species In *M Rossii* the filament is as long as the leaflet and there is scarcely any notching where the two join In N *Theobaldi* the notching is well marked (Fig 57B)

3 The filament is very short a mere spike like process

N *Stephensi*
N *maculata*
N *Theobaldi*
N *maculipalpis*

The Stigmatic Syphon — The eighth segment bears the stigmatic opening This is a large quadrilateral space with hard comb like chitinous processes on either side These have the teeth projecting backwards and are capable of being approximated so as to obliterate the cavity Into the anterior portion of the space under cover of a lip like process the two main air tubes open

The ninth segment is cylindrical in shape and is chiefly notable from the fact that it carries four large transparent papillae well supplied with air tubes and certain long curved hairs Of the hairs one series projects downwards so as to resemble a rudder The others project posteriorly There does not appear to be much variation in the different species

EXAMINATION OF THE LARVA

1 Some features e g feeding are conveniently studied by placing the larvae in a drop of water in a watch glass

For examining under a high power the activity of the larva must be restrained by a cover glass

3 Permanent preparations may be made at once by placing in strong formalin, then alcohol, then oil of cloves, then balsam (*vide* p 250)

4 Beautiful preparations of the palmate hairs etc are got by mounting the larval skeleton thrown off at the time of pupation

Pupation

Just before this process the larva becomes quieter The attitude also frequently alters becoming a hanging one, somewhat like that of a *Culex* larva

In this condition larvae are very readily killed by agitating the water (and it is difficult to carry larvae in this stage without killing them)

The change into the nymph is very sudden A few rapid motions and the larval skin is cast off leaving the characteristic nymph

The Nymph

This stage in the tropics usually lasts about forty eight hours When first the larval coat is cast the nymph is light in colour and may be readily overlooked Later, the nymph becomes darker and towards the end and immediately prior to the emergence of the imago *silvery patches* due to collections of air are seen beneath the cuticle

Pupae taken out of the water and kept on moist blotting paper will still develop into winged insects (Nuttall and Shipley) For the distinction between *Anopheles Culex* and other nymphae *vide* p 89 No differences have been described between the nymphae of the different species of *Anopheles*

Chapter XX

THE BREEDING PLACES OF ANOPHELES

Breeding Places of Anopheles

Directions for the collection of *Anopheles* larvae have already been given.

It is a matter of considerable importance to know what species breed in any particular situation. Larvae should be sought for in the most diverse situations and, after being examined and described, be allowed to hatch out. New species of *Anopheles* are often obtained in this way. It is the case in India, and almost certainly will be found to be so in other countries, that certain kinds of breeding places are preferred by certain species. A collection of larvae made from shallow puddles will be found to yield quite a different set of species to one made from a streamlet or pool full of vegetation, even though close to the puddles (Fig. 58).

The following tabular statement gives the more common situations of *Anopheles* breeding places and the species, as far as known, found in each. It is obvious that a great deal of work yet remains to be done.

1. Foul puddles near habitation — M. Rossii
2. Clean puddles without much algae and often turbid with suspended matter

(a) Pits and puddles near houses — M. Rossii, [illegible]

(b) Roadside puddles	N maculata P costalis
(c) Cattle footmarks	N Stephensi
(d) Shallow muddy sheets of water	M Rossii
(e) Pools in sandy river beds (f) Large pools in quarries etc	M culicifacies M Turkhudi
3 Puddles and pools with much alga Common in stream beds water trickling over rocks	N Fuliginosus N maculata
4 Earthenware vessels empty paraffin tins boats water barrels	N Stephensi P costalis
5 Wells springs	N Stephensi P costalis A Lindesayii
6 Swamps	
(a) Deep water with much aquatic vegetation	M Sinensis M barbirostris M paludis
(b) Rice fields wet cultivation of all kinds	M Rossii J Jeyporensis N maculata N maculipalpis A Lindesayii
7 Running water	
(a) Swiftly flowing streams	M Listoni
(b) Sluggish irrigation channel ditches muddy trickles edges of rivers	M Listoni M culicifacies N maculipalpis
(c) With much weed and alga	M Sinensis M barbirostris N fuliginosus N maculata N Theobaldi
(d) Stony and shallow	M culicifacies N Theobaldi M Turkhudi

8 Lakes with weedy margins — fuliginosus
9 Hill species — Lindesayii, maculata, gigas

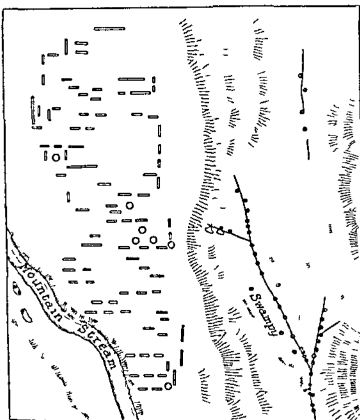


Fig 58 Portion of Coolie Lines on a Tea Plantation to show different breeding places of M. fossil M. listoni and N. maculata

○ = M. fossil ● = M. listoni and N. maculata

1

(b) Roadside puddles	N maculata P costalis
(c) Cattle footmarks	N Stephensi
(d) Shallow muddy sheets of water	M Rossii
(e) Pools in sandy river beds (f) Large pools in quarries etc	M culicifacies M Turkhudi
3 Puddles and pools with much alga Common in stream beds water trickling over rocks	N Fuliginosus N maculata
4 Earthenware vessels empty paraffin tins boat water barrels	N Stephensi P costalis
5 Wells springs	N Stephensi P costalis A Lindesayi
6 Swamps	
(a) Deep water with much aquatic vegetation	M Sinensis M barbirostris M paludis
(b) Rice fields wet cultivation of all kinds	M Rossii P Jeyporensis N maculata N maculipalpis A Lindesayi
7 Running water	
(a) Swiftly flowing streams	M Listoni
(b) Sluggish irrigation channels ditches muddy trickles edges of river	M Funesta M culicifacies N maculipalpis
(c) With much weed and alga	M Sinensis M barbirostris N fuliginosus N maculata N Theobaldi
(d) Stony and shallow	M culicifacies N Theobaldi M Turkhudi

8 Lakes with weedy margins	N fuliginosus
9 Hill pieces	N fund sayii
	N maculata
	N gigas

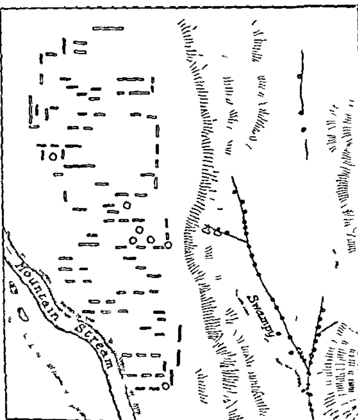


Fig 58 I rti n f C li I nes n n T i I lantit n t h t different bre ling pl es) M I n M I rmi ard N Ml ulata
⊂ = M Rossii ● = M Li t ns and N Mu ula i

I

Chapter XXI

THE IDENTIFICATION OF ANOPHELES LARVAE

1 *Naked Eye Characters* —Some larvae may be identified by the naked eye The distinction however, between most species is insufficient to allow of separation by this means

2 Observe that the colour of larvae is not dependent on species but on the nature of the food amount of light they have been exposed to in nature the colour of the water and other general conditions

3 The most distinctive of *Anopheles* larvae are those of *M Sinensis* and *M barbirostris* These are very large larvae most frequently black or black speckled with white but also brown or vivid green in colour One of their characteristics is a peculiar stick like appearance and the assumption of a bent or contorted attitude

The larvae of *M Turkhudi* can be detected by their attitude which is almost *Culex* like Larvae about to change into nymphae however also frequently adopt this position

Naked eye examination always requires verification by the microscope

(A) Larvae may be bred from ova deposited by females of a known species To successfully accomplish this requires a good deal of care

1 Remove the paper upon which the ova have been laid (p 96) and place in a small bottle containing some filtered fresh water from a pool or rain puddle

2 Place in a good light but take care that the sun by the focussing action of the glass does not heat the water otherwise the larvae will be killed

3 When the larvae are hatched transfer them (after a day or two) to a larger vessel of fresh water containing some weed When the fresh natural appearance of the water disappears more fresh water from a pool should be added

4 By keeping larvae in a not too porous earthenware vessel they may be placed with impunity all day in the direct sun It is necessary however to watch carefully to guard against desiccation and consequent death of the larvae

Larvae kept in flat partially glazed earthenware vessels with a certain amount of mud and placed in the sun develop more quickly than those kept in bottles

It is of course necessary to make certain that foreign ova or young larvae are not introduced with the fresh water

Some larvae are exceedingly difficult to rear artificially notably those of *M barbirostris* and *M Sinensis* They remain for long periods without perceptibly increasing in size and frequently die

(B) An alternative and less tedious way is to examine nearly adult larvae found in nature and to observe after accurately noting the larval characters what species of *Anopheles* eventually hatches out

Chapter XXI

THE IDENTIFICATION OF ANOPHELES LARVAE

1 *Naked Eye Characters*—Some larvae may be identified by the naked eye The distinction however between most species is insufficient to allow of separation by this means

2 Observe that the colour of larvae is not dependent on species but on the nature of the food amount of light they have been exposed to in nature the colour of the water and other general conditions

3 The most distinctive of *Anopheles* larvae are those of *M Sinensis* and *M barbirostris* These are very large larvae, most frequently black, or black speckled with white, but also brown or vivid green in colour One of their characteristics is a peculiar 'stick like' appearance, and the assumption of a bent or contorted attitude

The larvae of *M Turkhudi* can be detected by their attitude which is almost *Culex* like Larvae about to change into nymphae, however also frequently adopt this position

Naked eye examination always requires verification by the microscope

(A) Larvae may be bred from ova deposited by females of a known species To successfully accomplish this requires a good deal of care

1. Remove the paper upon which the ova have been laid (p. 96) and place in a small bottle containing some filtered fresh water from a pool or rain puddle.

2. Place in a good light, but take care that the sun, by the focussing action of the glass, does not heat the water, otherwise the larvae will be killed.

3. When the larvae are hatched transfer them (after a day or two) to a larger vessel of fresh water containing some weed. When the fresh natural appearance of the water disappears more fresh water from a pool should be added.

4. By keeping larvae in a not too porous earthenware vessel they may be placed with impunity all day in the direct sun. It is necessary, however, to watch carefully to guard against desiccation and consequent death of the larvae.

Larvae kept in flat partially glazed earthenware vessels with a certain amount of mud and placed in the sun develop more quickly than those kept in bottles.

It is of course necessary to make certain that foreign ova or young larvae are not introduced with the fresh water.

Some larvae are exceedingly difficult to rear artificially, notably those of *M. barbirostris* and *M. Sinensis*. They remain for long periods without perceptibly increasing in size and frequently die.

(B) An alternative and less tedious way is to examine nearly adult larvae found in nature and to observe, after accurately noting the larval characters, what species of *Anopheles* eventually hatches out.

By examining the larva on a slide without a coverglass the main characters may be noted without in any way damaging the larva which later becomes a nymph and eventually an imago. As a rule however many specimens of the same species are found together. By a preliminary examination larvae shewing the same characters may be sorted out and some specimens afterwards mounted and subjected to a more detailed examination whilst the rest are allowed to hatch out in due course.

The characteristics of the larvae which are of specific importance are, as we have seen—

1. The antennae
2. The clypeal hairs
3. The leaflets of the palmate hairs
4. The segments carrying palmate hairs

By means of these characters most species of *Anopheline* larvae can be identified. So far as Indian *Anophelines* are concerned the following characters hold good —

Type 1.—Larvae with the external pair of clypeal hairs converted into a cockade like tuft (Fig. 56 C).

Species having larvae of this type are—

M. barbirostris

M. Sinensis sub sp. *nigerrimus*

Larvae of this type also have a large branched hair upon the antenna and the leaflets of the palmate hairs differ markedly from all other larvae (Fig. 57 B).

Type 2.—Larvae with the external frontal

hairs branched but not developed into tufts (Fig 56) *N fuliginosus*
Ce pulcherrimus

Type 3 —Larvae with the external pair of frontal hairs simple and unbranched and with palmate hairs on every abdominal segment and on the thorax (Fig 56)

M culicifacies
M Listoni

Type 4 —Larvae with the external pair of frontal hairs simple and unbranched but with no developed palmate hairs on thorax or first abdominal segment (Fig 56)

M Rossii
N Stephensi

Type 5 —Larvae with two large additional hairs placed behind those already mentioned Also with first three abdominal segments free from palmate hairs *M Turkhudi*

Larva of M barbirostris — Antenna with large branched hair External pair of frontal hairs developed into cockades Palmate hairs on second to seventh abdominal segments Leaflets of palmate hairs lanceolate in shape and deeply serrated in outer half Head of larva without pigmented markings

Larva of M Sinensis — Antenna with large branched hair External pair of frontal hairs developed into cockades Palmate hairs (?) Leaflets of palmate hairs lanceolate with serrations in outer half Head of larva without pigmented markings

By examining the larva on a slide without a coverglass the main characters may be noted without in any way damaging the larva, which later becomes a nymph and eventually an imago. As a rule, however, many specimens of the same species are found together. By a preliminary examination larvae shewing the same characters may be sorted out and some specimens afterwards mounted and subjected to a more detailed examination whilst the rest are allowed to hatch out in due course.

The characteristics of the larvae which are of specific importance are, as we have seen—

1. The antennae.
2. The clypeal hairs.
3. The leaflets of the palmate hairs.
4. The segments carrying palmate hairs.

By means of these characters most species of *Anopheles* larvae can be identified. So far as Indian *Anopheles* are concerned, the following characters hold good:—

Type 1.—Larvae with the external pair of clypeal hairs converted into a cockade-like tuft (Fig. 56).

Species having larvae of this type are—

M. barbirostris.

M. sinensis sub. sp. *nigerrimus.*

Larvae of this type also have a large branched hair upon the antenna, and the leaflets of the palmate hairs differ markedly from all other larvae (Fig. 57B).

Type 2.—Larvae with the external frontal

hairs branched but not developed into tufts (Fig 56) *N fuliginosus*
Ce pulcherrimus

Type 3—Larvae with the external pair of frontal hairs simple and unbranched and with palmate hairs on every abdominal segment and on the thorax (Fig 56)

M culicifacies
M Listoni

Type 4—Larvae with the external pair of frontal hairs simple and unbranched but with no developed palmate hairs on thorax or first abdominal segment (Fig 56)

M Rossii
N Stephensi

Type 5—Larvae with two large additional hairs placed behind those already mentioned Also with first three abdominal segments free from palmate hairs *M Turkhudi*

Larva of M barbirostris — Antenna with large branched hair External pair of frontal hairs developed into cockades Palmate hairs on second to seventh abdominal segments Leaflets of palmate hairs lanceolate in shape and deeply serrated in outer half Head of larva without pigmented markings

Larva of M Sinensis — Antenna with large branched hair External pair of frontal hairs developed into cockades Palmate hairs (?) Leaflets of palmate hairs lanceolate with serrations in outer half Head of larva without pigmented markings

By examining the larva on a slide without a coverglass the main characters may be noted without in any way damaging the larva, which later becomes a nymph and eventually an imago. As a rule, however, many specimens of the same species are found together. By a preliminary examination larvae shewing the same characters may be sorted out and some specimens afterwards mounted and subjected to a more detailed examination whilst the rest are allowed to hatch out in due course.

The characteristics of the larvae which are of specific importance are, as we have seen—

1. The antennae.
2. The clypeal hairs.
3. The leaflets of the palmate hairs.
4. The segments carrying palmate hairs.

By means of these characters most species of *Anopheles* larvae can be identified. So far as Indian *Anopheles* are concerned, the following characters hold good:—

Type 1.—Larvae with the external pair of clypeal hairs converted into a cockade like tuft (Fig. 56).

Species having larvae of this type are—

M. barbirostris.

M. Sinensis sub sp. *nigerrimus.*

Larvae of this type also have a large branched hair upon the antenna and the leaflets of the palmate hairs differ markedly from all other larvae (Fig. 57B).

Type 2.—Larvae with the external frontal

hairs branched but not developed into tufts (Fig. 56) *A. fuliginosus*
Ce. pulcherrimus

Type 3.—Larvae with the external pair of frontal hairs simple and unbranched and with palmate hairs on every abdominal segment and on the thorax (Fig. 56)

M. culicifacies
M. Listoni

Type 4.—Larvae with the external pair of frontal hairs simple and unbranched but with no developed palmate hairs on thorax or first abdominal segment (Fig. 56)

M. Rossii
N. Stephensi

Type 5.—Larvae with two large additional hairs placed behind those already mentioned. Also with first three abdominal segments free from palmate hairs. *M. Turkhudi*

Larva of M. barbirostris.—Antennae with large branched hair. External pair of frontal hairs developed into cockades. Palmate hairs on second to seventh abdominal segments. Leaflets of palmate hairs lanceolate in shape and deeply serrated in outer half. Head of larva without pigmented markings.

Larva of M. Sinensis.—Antennae with large branched hair. External pair of frontal hairs developed into cockades. Palmate hairs ( ). Leaflets of palmate hairs lanceolate with serrations in outer half. Head of larva without pigmented markings.

The habits of both these species, *M barbirostris* and *M Sinensis* sub sp *nigerrimus*, are very similar The larvae are found in water with much aquatic vegetation—rivers, lakes, ponds, and swamps They are only caught singly, but are generally widespread in their occurrence where large bodies of water are present

Larva of N fuliginosus—Antenna without large branched hair External pair of frontal hairs branched (branches usually six in number) Palmate hairs on second to seventh abdominal segments Leaflets of palmate hairs with very marked shoulder at origin of terminal filament Terminal filament from one half to two thirds the length of basal portion Head of larva with distinctive markings

Larva of M culicifacies—Antenna without large branched hair Frontal hairs all unbranched Palmate hairs on first to seventh abdominal segments and a pair of fairly developed ones upon the thorax *Palmate hairs with terminal filament* nearly as long as basal portion Head with markings

Larva of M Listoni—Antenna without large branched hair Frontal hairs simple Palmate hairs on all segments and very well developed pair on thorax The palmate hairs in this species are very large The terminal filament is nearly as long as basal portion

Larva of Ce pulcherrimus—Antenna without large branched hair Outer pair of frontal hairs branched (six branches) Palmate hairs on second to seventh abdominal segments Filament of palmate hair nearly as long as basal portion Head markings present

Nature of breeding place unl nown

Larva of M Rossii —Antenna without large branched hair Frontal hairs unbranched Palmate hairs second to seventh abdominal segments Terminal filament of palmate hair very long often longer than basal portion The shoulder at the origin of the filament is very slightly marked There are markings upon the head (Fig 57A)

Breeds nearly always in small pools near houses These pools are frequently foul and nearly always muddy The female lays her eggs very readily in captivity

Larva of N maculipalpis —Antenna without large branched lateral hair Frontal hairs are peculiar and show a condition intermediate between the branched hairs of *M barbirostris* *N fuliginosus* and the unbranched hairs of *M Rossii* and other species (Fig 56) Palmate hairs on second to seventh segments Leaflets of palmate hairs have very short filaments The notching at the termination of the leaflet is not so marked as in *N Theobaldi*

Larva of N Theobaldi (GILES) —Antenna without large branched lateral hair Frontal hairs unbranched Palmate hairs on second to seventh segments Leaflets of palmate hairs have very short filaments There are marked notches at the ending of the leaflet in the filament (Fig 57B)

The larvae of this species frequent especially sluggish streams with much growth of alga They were found by us in Nagpur in association with *N fuliginosus M barbirostris* and *M Listoni*

The habits of both these species *M barbirostris* and *M Sinensis*, sub sp *nigerrimus*, are very similar The larvae are found in water with much aquatic vegetation—rivers, lakes ponds and swamps They are only caught singly, but are generally widespread in their occurrence where large bodies of water are present

Larva of N fuliginosus —Antennæ without large branched hair External pair of frontal hairs branched (branches usually six in number) Palmate hairs on second to seventh abdominal segments Leaflets of palmate hairs with very marked shoulder at origin of terminal filament Terminal filament from one half to two thirds the length of basal portion Head of larva with distinctive markings

Larva of M culicifacies—Antennæ without large branched hair Frontal hairs all unbranched Palmate hairs on first to seventh abdominal segments, and a pair of fairly developed ones upon the thorax Palmate hairs with terminal filament nearly as long as basal portion Head with markings

Larva of M Listoni—Antennæ without large branched hair Frontal hairs simple Palmate hairs on all segments and very well developed pair on thorax The palmate hairs in this species are very large The terminal filament is nearly as long as basal portion

Larva of Ce pulcherrimus—Antennæ without large branched hair Outer pair of frontal hairs branched (six branches) Palmate hairs on second to seventh abdominal segments Filament of palmate hair nearly as long as basal portion Head markings present

Nature of breeding place unknown.

Larva of M. Rossii.—Antennæ without large branched hair. Frontal hairs unbranched. Palmate hairs second to seventh abdominal segments. Terminal filament of palmate hair very long, often longer than basal portion. The shoulder at the origin of the filament is very slightly marked. There are markings upon the head (Fig. 57A).

Breeds nearly always in small pools near houses. These pools are frequently foul and nearly always muddy. The female lays her eggs very readily in captivity.

Larva of N. maculipalpis.—Antenna without large branched lateral hair. Frontal hairs are peculiar and show a condition intermediate between the branched hairs of *M. barbirostris*, *N. fuliginosus* and the unbranched hairs of *M. Rossii* and other species (Fig. 56). Palmate hairs on second to seventh segments. Leaflets of palmate hairs have very short filaments. The notching at the termination of the leaflet is not so marked as in *N. Theobaldi*.

Larva of N. Theobaldi (GILES).—Antenna without large branched lateral hair. Frontal hairs unbranched. Palmate hairs on second to seventh segments. Leaflets of palmate hairs have very short filaments. There are marked notches at the ending of the leaflet in the filament (Fig. 57B).

The larvæ of this species frequent especially sluggish streams with much growth of algæ. They were found by us in Nagpur in association with *N. fuliginosus*, *M. barbirostris* and *M. Listoni*.

Larva of M Turkhudi —The larva is *Culex* like in some of its characters though undoubtedly much more nearly related to the *Anopheles* type

The full grown larva is distinguished by the adoption of the slightly hanging attitude The chief characters of the larva are —

1 Two large additional frontal hairs are developed which reach as far forward as the longest of the hairs described in other larvae

2 The shape of the head differs from that of the ordinary *Anopheles* larva

3 The palmate hairs are only represented on two or *three* abdominal *segments* namely, the fourth fifth and sixth They are absent on the first three abdominal segments

4 The palmate hairs are small and poorly developed The leaflets are irregular and the terminal filament blunt

This species must be looked upon as a form which in its egg and larval stages has lost many of the characteristics of *Anopheles* eggs and larvae and has approached in these stages the characters of the eggs and larvae of *Culex*

Classification of Indian Anophelfs According to Larval Characteristics *

A bra h d h l il	Y r d f h l tnl		M Sinensis	Leaflets of palmate hairs lanceolate and serrated
			M barbirostris	Ditto
	S mpl f l		M Lindesayii	Leaflets of palmate hairs shewing regular and deep notching (Fig 57B)
ae with br hed l er l h lr	Simpl bra hed typeal h t	Filam fle fl l K	M Rossii	Palmate hairs well developed on third to seventh segments
			M Culicifacies	Palmate hairs on all segments and on thorax
			M Listoni	Palmate hairs very large on all segments and on thorax
		Fil m h of l fl	N Stephensi N maculata N Theobaldi	
	Fea hered typeal h l	Filam h	N maculipalpis	
		Fil me l fl	P Jeyporensis	Palmate hairs very large on all segments and thorax
	B rched t pe l h l		N fuliginosus C Pulcherrimus	Filaments of leaflets long
	Tw b nd	pea lor La fl pk.o	M Turkhudi	Palmate hairs on fourth fifth and sixth segments only filament short leafl t rudimentary

* Th L rv f th m g l d p es f d cribed

To Mount Larvae

1 Place a drop of formalin in a hollow ground slide The drop must be just sufficient to fill the cell when the coverglass is in position

By means of a pipette or spoon take up a larva and removing the excess of water, allow the larva to float off into the drop of formalin

Place the coverglass in position, avoiding air bubbles, and ring with Canada balsam, etc

It is important that no air bubbles are included as a white deposit forms around them

If too much formalin has been added, the excess must be carefully removed before ringing If hollow ground slides are not available a ring of balsam may be made on the slide and allowed to become somewhat hard Fill the cavity with formalin place the larva therein, and cover carefully with a coverglass Avoid excess of fluid or air bubbles It is best to allow the Canada balsam to be just soft enough to stick to the coverglass

Larvae mounted in this way retain their characters very well, and the clypeal and palmate hairs can be examined with ease

2 *To Mount in Balsam* —If placed in alcohol oil of cloves or xylol and balsam in the ordinary way the shrinkage of the soft parts and even of the hairs is very great

On no account touch the larvae with forceps, and only occasionally, and with the utmost care with a needle point

Place a number of larvae in a covered watch glass containing formalin Leave for twenty four hours at least

Lift each larva carefully by means of a strip of cigarette paper. Drain off the excess of formalin, and place with the greatest care in absolute alcohol. Allow the specimen to remain for at least ten minutes in alcohol.

Remove with cigarette paper to a watch glass containing oil of cloves. With cigarette paper transfer to a slide. Remove excess of oil of cloves, mount in a large drop of balsam, taking care that the dorsum of the larva is upwards.

If great care is taken not to detach the hairs by handling, the larval characters are beautifully displayed in this way.

It is well known in a general way that in one country malaria is more intense than in another, but here we have a means of exactly measuring this difference, and moreover, in the different parts of any particular district. We may illustrate this by the differences we found in Bengal in an extent of country where as far as we could judge the climatic conditions were practically identical yet we find in the environs of Calcutta the endemic index is o while in the Duars (at the foot of the Himalayas) it is as high as seventy two (Fig. 59). We found however, that there was one important matter in which the Duars differed from Calcutta, and that was in its *Anopheles* fauna. Whereas in Calcutta *M. Rossii* was the predominant species in the Duars *M. Listoni* was the commonest *Anopheles*.

Again in the Jeypore district (Madras), we had a district of uniformly high endemic index, fifty to one hundred and here we found an *Anopheles P. Jeyporensis* which we had not encountered elsewhere so that the view seemed tenable that the high endemicity of these districts was dependent on their special *Anopheles* fauna. To test to what extent species was concerned in determining endemicity we then made use of another more exact method, viz., determining by dissection whether any difference occurred amongst the different species in the percentage of infected specimens. we were able to carry this out in the case of *M. Rossii* and *M. culicifacies*. We caught these species in the same huts in the same villages at the same time and determined by actual dissection the percentage of glands infected with sporozoits. The results were most striking, and

fully confirmed our previous idea based on more general considerations of the importance of species. They were as follows —

I. MIAN MIR (PUNJAB)

	Number dissected	Number with sporozoites	Per cent
M. Culicifacies	259	12	4.6
M. Rossii	492	0	0

II. ENNUR (MADRAS)

	Number dissected	Number with sporozoites	Per cent
M. Culicifacies	69	6	8.6
M. Rossii	364	0	0

Undoubtedly then under natural conditions the species is here a very important factor.

Again under artificial conditions (feeding experiments) we found that there was a difference in the number of zygotes found in the stomach as the result of feeding.

The species which appeared to be most active were —

M. culicifacies
N. Stephensi
N. Theobaldi

Those in which zygote formation seemed less abundant were —

M Rossii
M Turkhudi
M barbirostris

It should be noted however, that in these experiments *M Rossii* became infected while in nature it has never been found infected by us

There are moreover, many considerations which lead to the conclusion that in nature all species of *Anopheles* are not equally concerned in the transmission of malaria

We may have countless numbers of *M Rossii*, as in Calcutta (environs) and get a malarial index of 0 and this appears to hold good in Madras Bombay and as far as our observations go universally On the other hand, where we find *M Listoni M culicifacies, P Jeyporensis* in India we have a high endemic index

The group of mosquitoes, those associated with intense malaria are small dark mosquitoes with unbanded legs (*Myzomyia* group 1)

M Funestus and P Costalis in Africa

The former mosquito is like *M Listoni*, which it closely resembles a *breeder in clean* waters *streams springs etc*, while *P costalis* is found breeding in shallow pools about houses and frequents towns (in Africa) which *M funestus* does not

M funestus was found by us to be infected in the Lagos hinterland to the extent of twenty five to fifty per cent

P costalis, in Lagos itself contained only three per cent of sporozoits

It is important then to determine precisely the species in a district and to determine the percentage of infection with sporozoits

LITERATURE

Stephens and Christophers *Malarial Reports to the Royal Society* Series VI and VII Harrison and Son London

Chapter XXIII

TO MAKE A MALARIAL SURVEY

Endemic Malaria

The clue to the epidemiology of malaria in the tropics is to be found in the infection of the native population of a country. The malaria of Europeans is merely the result of their exposure to infection from this source. Investigation into the natural history of malaria, therefore, resolves itself largely into the study of native or endemic malaria. It has always been recognized that in a particular country certain districts are more malarial than others. It was not, however, till Koch used the percentage of infected children as the test of the malarial intensity of a place that accurate measurement of this became possible.

To Investigate the Endemic Malaria of a District

(A) *The Breeding Places of Anopheles*—

1. Examine all collections of water within half a mile. Stir up the mud of small puddles and use a dipper where the water is weedy or difficult of access. Examine wells, 'chatties,' streams and swamps, as well as pools of every description. Take specimens of larvae from each, placing in specimen tubes and labelling.

2 Determine the species of the larvae collected

3 Make a map of the neighbourhood, noting—

(a) All breeding grounds

(b) What species are found breeding in those examined (Fig 60)

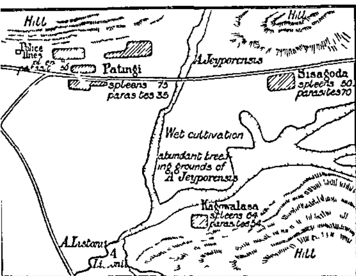


Fig 60 Map showing how to make a Malarial Survey

(B) *The Presence of Winged Anopheles*—

1 Search in outhouses under eaves etc as described in Chap XIII for *Anopheles* Determine the species note relative numbers of each species on map The relation of *Anopheles* to native dwellings will probably be evident

2 In the dry season the search for *Anopheles* may be negative and there may be no breeding places Make in the most sheltered places small cement pools and keep these filled with water After a certain number of days they may contain young *Anopheles* larvae if the adults are present in the houses (It is necessary to be sure one's water supply does not contain young larvae or eggs) The absence of the larvae in the pools does not necessarily mean however that adult *Anopheles* are not present in the houses (see choice of breeding grounds by different species of *Anopheles*)

Note the result in the case of each test pool

3 In the conditions just described observe the pools made by the first shower of rain of the on coming rains Note after three days have passed the presence of larvae in many of these Note the presence of these on the map The distribution of *Anopheles* at the end of the dry season will usually be found to correspond to that of native huts

The Prevalence of Malaria

If we proceed to ascertain to what extent malaria prevails in a district we may attempt to do so in several ways

1 We may consult hospital statistics and returns of death from malaria This method is open to such grave error that it is extremely doubtful whether it is worth the labour bestowed upon it

2 We may determine to what extent en largement of the spleen occurs This method has been largely used

PRECAUTIONS NECESSARY IN APPLYING THE SPLEEN TEST

1. *The Age of the Individuals Examined.*—The enlargement of the spleen due to ordinary malarial infection tends to disappear once the individual has ceased to suffer from malarial infection. In very malarious countries where each individual after childhood has become highly immune the adult population usually shew no splenic enlargement (Tropical Africa).

In less malarious regions the adults have not become highly immunized and a certain number of them will be found with enlarged spleens and malarial infection. The use then of the percentage of adults with enlarged spleens is not a reliable method of determining the real intensity of malaria.

In children the spleen enlargement appears to require a certain time to become apparent and it takes a certain time to disappear as the malarial infection disappears with ensuing immunity.

In the examination of children for splenic enlargement and the presence of parasites in their blood we found:

(i) In the early ages one to two years the number infected is usually in excess of those shewing splenic enlargement.

(ii) Above two years the spleen rate is usually somewhat in excess of the parasite rate.

(iii) Above ten years the spleen rate is usually considerably in excess of the parasite rate.

In the use of a spleen census one should then avoid a mixed adult and child count and children

between two years and ten years of age should be chosen

2 *The District in Question*—It seems clear that the comparison of the malaria of widely different regions by means of the percentage of enlarged spleens in the children is not possible We have however found that in Bengal, the parasite rate and the spleen rate in children varied proportionally, the spleen rate was, however, nearly always about double that of the parasite rate

3 *Time of Year Seasonal Variations*—We may determine by actual blood examination how many individuals have parasites in the peripheral circulation By the use of the parasite rate in children up to ten years of age we get a definite and true index of endemicity which may be used in the comparison of one locality with another

4 To the *last method* we would add as a complimentary one the determination of the percentage of infected *Anopheles* as giving the actual risk of infection in a district

THE DETERMINATION OF THE ENDEMIC INDEX OF A PLACE

1 Place a number of cleaned slides in a slide box Take a straight surgical needle paper and pencil

2 Choose any village or quarter of a town Get the assistance of a native with local influence the native magistrate in an Indian bustee, the chief in an African village Instruct him to muster the children of the village The free display of 'pice half pence, etc, will greatly aid one,

and by palpating a few spleens previously to taking blood specimens the children will come readily. It is well first to take the blood of one or two adults or big boys so as to allay fears. In all cases it will be found best to take for granted the willingness of the child and if the operation is quickly and quietly performed there is little objection, especially when each receives payment.

3. Make dry blood films by the method described in the early part of the book.

4. At the same time a spleen census may with advantage be made.

On examining the films determine —

(i) Number shewing parasites or pigmented leucocytes in the blood.

(ii) The species of each parasite present and the percentage value for each if the numbers are large enough.

To Determine the Infection in the Anopheles

(The Sporozoite Rate)

1. Collect as large a number of *Anopheles* as convenient from the village in and around which the previous observations have been made.

2. Dissect as many specimens as possible, noting in each case the species dissected and noting in which species, if any, sporozoits are found.

In many cases the sporozoit rate is extraordinarily low, e.g. two per cent, although *Anopheles* are abundant and the malarial index

is not low In others, especially in African bush stations the percentage may reach fifty per cent

3 Leave specimens not dissected for several days and examine the mid gut for zygotes

Malarial Infection of Europeans

Although malaria is an infectious disease and can arise only from an original human source yet in the tropics we can no longer consider the origin of infection as occasional and due to the presence of other cases of fever In the tropics and especially in Africa we are dealing with a disease which is a normal condition of childhood and which with the coincident infection of *Anopheles* is the usual accompaniment of every native hut

European malaria in the tropics is indeed chiefly dependent on two factors—

1 *The degree of exposure to native malaria* *i e* the proximity to native dwellings

2 The endemic index of the native dwellings in question

To Investigate European Malaria

1 Examine the blood of as many Europeans as possible *Enquire carefully* whether the person is taking quinine at the time, also take the temperature

(i) The number shewing parasites or crescents

(ii) The presence of pigmented leucocytes

(iii) The presence of an increase of the large mononuclear leucocytes

In every case make a differential count of the leucocytes and keep the record

Observe especially any community of Europeans shewing a larger percentage than usual of malarial infection Note the conditions under which these are living and note also the probable greater prevalence of blackwater fever in these communities e g Roman Catholic Fathers West African miners railway communities Europeans in poor circumstances living in the slums of native towns etc Syrian hawkers etc Note those communities habitually taking quinine

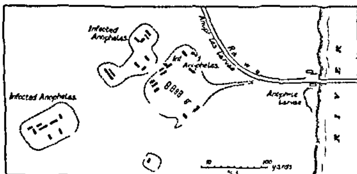


Fig 61 Sketch to illustrate European infection with Malaria from the native children

2 Note the usual relation between the degree of ill health and the proximity of native huts Make a map shewing European dwellings and shewing huts and hovels in relation with these (Fig 61)

3 Make a thorough investigation of the conditions in these huts

(i) The percentage of infected children in each group

(ii) The degree of infection of the adults

(iii) Roughly estimate the number of *Anopheles* present whether swarming, abundant, scanty, *or impossible to detect by search In the latter* case make several test pools

(iv) Determine the species present and the relative numbers of each

(v) Determine the sporozoit rate for each species

(vi) Carefully map all breeding places, noting what larvae are found

4 Capture as many *Anopheles* as possible in the European houses especially in the morning, and by looking within the nets Determine the species *sporozoit rate*, and *from* where probably derived Examine the ovaries and spermatheca and note whether freshly hatched or impregnated females are chiefly found Note the presence or absence of males

In investigating the malaria of any such settlement native and European continue the observations if possible throughout the year Make observations on—

1 Seasonal variations in the endemic index (percentage of infected children)

2 Seasonal variations in the number of cases among Europeans

3 Prevalence of any particular species of *Anopheles* at any time of the year

4 Distance of flight of *Anopheles* from breeding grounds, etc

5 Sporozoit rate of *Anopheles* at different times of the year

6. Examine especially the conditions where *Anopheles*, breeding places, native huts, opportunity for constant importation of malaria and numerous susceptible children exist and yet there is a complete absence of endemic malaria. In Africa it will probably be impossible to find such places, but they occur in India.

Endemic Areas of a Country

The map (p. 253) shews how the endemicity of large areas of a country is a very variable one. When opportunity offers the endemic index should be determined for each locality and as far as possible all the other facts detailed above. But the simple taking of the blood of a number of children (under ten) in any village gives at once valuable information as to malaria of the district, information which often is quite unsuspected. Thus as is shewn in the map the endemic index of Calcutta is 0, that is to say in the immediate environs (not in the town itself) where practically the condition is one of a number of isolated villages there is no malaria among the native children. At Jalpaiguri the figure is low, twelve per cent, but on reaching the foot of the Himalayas we find the extremely high figure seventy two per cent. In this case we were able, among other differences, to find a different species of *Anophele* which as we have seen is undoubtedly an important factor.

In other cases however all the conditions may be apparently identical but within a distance of even ten miles we may get a change from an endemic index of 0 (Madras) to ninety (Ennur).

(i) The percentage of infected children in each group

(ii) *The degree of infection of the adults*

(iii) Roughly estimate the number of *Anopheles* present, whether swarming abundant, scanty, or impossible to detect by search In the latter case make several test pools

(iv) Determine the species present and the relative numbers of each

(v) Determine the sporozoit rate for each species

(vi) Carefully map all breeding places, noting what larvae are found

4 Capture as many *Anopheles* as possible in the European houses especially in the morning and by looking within the nets Determine the species sporozoit rate, and from where probably derived Examine the ovaries and spermatheca, and note whether freshly hatched or impregnated females are chiefly found Note the presence or absence of males

In investigating the malaria of any such settlement native and European continue the observations if possible throughout the year Make observations on—

1 Seasonal variations in the endemic index (percentage of infected children)

2 Seasonal variations in the number of cases among *Europeans*

3 Prevalence of any particular species of *Anopheles* at any time of the year

4. Distance of flight of *Anopheles* from breeding grounds, etc

5 Sporozoit rate of *Anopheles* at different times of the year

6 Examine especially the conditions where *Anopheles*, breeding places, native huts, opportunity for constant importation of malaria and numerous susceptible children exist and yet there is a complete absence of endemic malaria. In Africa it will probably be impossible to find such places but they occur in India.

Endemic Areas of a Country

The map (p 253) shews how the endemicity of large areas of a country is a very variable one. When opportunity offers the endemic index should be determined for each locality and as far as possible all the other facts detailed above. But the simple taking of the blood of a number of children (under ten) in any village gives at once valuable information as to malaria of the district, information which often is quite unsuspected. Thus as is shewn in the map the endemic index of Calcutta is 0, that is to say in the immediate environs (not in the town itself) where practically the condition is one of a number of isolated villages there is no malaria among the native children. At Jalpaiguri the figure is low, twelve per cent, but on reaching the foot of the Himalayas we find the extremely high figure seventy two per cent. In this case we were able among other differences to find a different species of *Anopheles*, which as we have seen is undoubtedly an important factor.

In other cases however all the conditions may be apparently identical, but within a distance of even ten miles we may get a change from an endemic index of 0 (Madras) to ninety (Ennur).

These differences hold good in other countries *e g* in Italy Here the mortality from malaria in the north is comparatively trifling while in the south and the islands it is severe

Here the difference may be due to differences in climate but this explanation does not suffice in the examples in India we have mentioned

Again we have great irregularities in the distribution of the species of parasite The quartan for instance in the Duars (Bengal) is exceedingly common amongst the native children *but in Lahore it is rare*

Similar differences have been noted in Algeria, where over large areas the quartan parasite is extremely rare yet in a few localities it occurs in seventy per cent of cases (BILLET)

So in India, as a whole we have certain small areas where malaria is intense *e g* the Duars Jeypore (Madras) and Kanara (Bombay) (CHRISTY) where we also find blackwater fever yet in others as in the Central Provinces, where apparently all the conditions are favourable we have only a moderate intensity

We require then to examine carefully the endemic indices over large areas in order to get an accurate idea of the variations in endemic malaria The instances we have given will shew how erroneous it is to say broadly such and such a country is highly malarial for while this may be true of one district it might be quite untrue of another

Further, after having established these broad data it will be necessary to make a close survey of each individual district in order to endeavour to explain the factors at work

Chapter XXII

CLINICAL STUDY OF MALARIA

EXAMINATION OF RED CELLS

In blood counting much practice only can give accurate results; inaccurate results are misleading and useless. For comparative purposes counts should be made always at the same time if possible, to obviate the effect of food, etc.

For diluting the blood 0.9 per cent. salt solution may be used, or preferably Toisson's fluid, which has the following formula:

H_2O	160 c.c.
Glycerin	30 c.c.
Sodium sulphate	8 grammes
Sodium chloride	1 gramme
Methyl violet (or other stain sufficient to colour the nuclei of the leucocytes)	

The diluting fluid must always be poured into a watch glass, and it should not be sucked up out of the stock solution.

No pressure must be used to make the blood drop exude from the finger.

Blood is then sucked up to the mark 1 on the pipette, and the end of the pipette carefully wiped before plunging it into the Toisson's fluid. The Toisson is sucked up to the mark 101 exactly. The pipette is then rotated between finger and thumb

so as to ensure thorough mixing After rejecting the first few drops which consist of diluting fluid simply, a small drop of the mixture is blown on to the counting chamber and the coverglass applied as rapidly as possible No fluid should escape into the moat or under the coverglass When NEWTON s rings are seen between the cover glass and side of the chamber, the former is in its right closely applied position

In counting the corpuscles in each square include of those that touch or overlap the sides, only those on the left hand and top side or right *hand and bottom side* *Count at least 1,000 cells,* the error is then only about two per cent

The number of corpuscles per mm³

$$= \frac{\text{No of corpuscles counted} \times \text{dilution } (100) \times 4\,000}{\textit{Number of squares counted}}$$

The normal average values are for man 5 000 000, for woman 4,500,000

TOTAL LEUCOCYTE COUNT

The leucocytes may also be counted at the same time as the red cells, *i e* from the pipette used for the red This method has the advantage that all errors effect both counts equally, and the true ratio of red to white may still be got Now for counting the leucocytes much larger fields are necessary than in the case of the red, as for every five hundred red cells there is *only one white* so that counting chambers especially ruled are often sold for this purpose but their use is unnecessary and may be obviated by the following method

We require simply to determine the area of the whole microscope field with a given eye piece objective and given length of tube

1 One division of a Thoma Zeiss square = 05 millimetre

2 So that if the diameter happened to cover eight divisions the diameter would be 4 millimetres or the radius 2 millimetres

3 The area of the field will therefore be πr and the corresponding volume of blood $\pi r \times \frac{}{1}$ ($- =$ depth between coverglass and chamber i e $\pi r = \frac{}{795}$ cubic millimetres

4 So that to get the number of leucocytes in the cubic millimetre we must multiply by 795 1 but it would be much more convenient to multiply by 100 In that case where $\pi r \times - = \frac{}{00}$ $r = 1\ 85$ millimetres or diameter of field 3 357 millimetres We require therefore to arrange our microscope so that the diameter is of this value and this is readily done

Two observations only are necessary

1 Draw the tube of the microscope out so that diameter of field covers exactly eight divisions of the Thoma Zeiss chamber note length of tube Let this = x

2 Draw tube out so that diameter covers exactly seven divisions Note length of tube = y

3 Therefore an increase in tube length of y x reduces diameter from eight divisions (4 millimetre) to seven divisions (35 millimetre) difference = 05 millimetre

4 Required to calculate what increase in length will cause reduction from 4 to 357 (difference = 043)

This will be $\frac{y - x}{05} \times 043$

This calculation is made once for all by the observer for his microscope and the tube is drawn out the required amount using of course the same eye piece and objective

To find total number of leucocytes per mm^3:—

$$\frac{\text{Total number counted} \times 100 \times \text{dilution } (100)}{\text{Number of microscope fields counted}}$$

Count one hundred if possible

The leucocytes may also be counted in the special pipette for white cells but here again the method of counting by using the whole microscope field should be used If the white counter is used the diluting fluid should be acetic acid 0 3 per cent Sufficient gentian violet or methyl violet is added to this to colour the nuclei

To Clean Pipettes

For any accuracy of observation the pipettes should be scrupulously clean There should not be the slightest tendency for the glass ball to stick to the sides After a count has been made the rubber tube is removed and the contents ejected by blowing from the pointed end

1 Suck up dilute acetic acid so that all traces of stain are removed

2 Suck up several lots of clean water to remove the acid

3 Then absolute alcohol two to three times to remove the water

4 Then ether two to three times to remove the alcohol

5 Finally blow hot air through with a syringe the glass barrel of which may be heated in a flame (or simply *suck* air through)

These procedures take a very short time, and it is a satisfaction to know that the pipette has been put away perfectly clean and ready for the next observation

THE ESTIMATION OF THE HAEMOGLOBIN

GOWERS's haemoglobinometer is the simplest and best. In sucking up the blood take care not to hold the tube too vertical, as the blood readily flows out from the rather large calibre of the tube. Order from a good maker, as several inferior instruments are on the market. The round form of tube is more easy to manipulate than the flat.

The standard of comparison in this apparatus is pikro carmine gelatine, the colour of which corresponds to a one per cent watery solution of normal blood.

All blood counting apparatus, etc., can be got from T. HAWKSLEY, 357 Oxford Street, London, W.

DARE's haemoglobinometer is accurate. It possesses the advantage of dispensing with a pipette. It costs £4.

TO COUNT PLATELETS

Diluting fluid: glycerine saturated with dahlia and two per cent saline solution, take equal parts of these.

The ratio of platelets to red cells is 1 : 8 about.
The absolute value per mm.^{3} 635,000 about.

Differential Counting of Leucocytes (*vide* page 41).

THE LEUCOCYTES IN MALARIA

We shall consider (1) the total leucocytes, (2) the percentage value of each kind.

The Total Leucocytes.—We may take 10,000 as the normal value per mm.^{3} and as 5,000,000

is the normal value for red cells, the proportion of white to red is

$$\frac{WC}{RC} = \frac{10\,000}{5\,000\,000} = \frac{1}{500}$$

Now in malaria we may find two conditions, either that the total number of leucocytes is considerably below the normal value 10,000, *i.e.* there is leucopenia or hypoleucocytosis or that the total number is much above 10,000, *i.e.* leucocytosis. If there is leucopenia say for instance, the total number is 5,000 instead of 10,000 then

$\frac{WC}{RC} = \frac{5\,000}{5\,000{,}000} = \frac{1}{1\,000}$ *i.e.* the fraction $\frac{WC}{RC}$ is smaller than normal.

If on the contrary the total leucocytes are 20,000 instead of 10,000 *i.e.* leucocytosis, then

$\frac{WC}{RC} = \frac{20\,000}{5\,000\,000} = \frac{1}{250}$ *i.e.* the fraction $\frac{WC}{RC}$ is greater than normal.

It is this ratio $\frac{WC}{RC}$ that it is important to determine, for unless the red cells are counted as well as the white little value attaches to the leucocytic value.

Turning now to malaria we find that we get changes of the following kinds —

(1) 11 a.m. rigor. Red cells = 2,900,000; White cells 10,000 — $\frac{WC}{RC} = \frac{1}{290}$

i.e. leucocytosis

(2) 11.30 a.m. *rigor completed* — $\frac{WC}{RC} = \frac{1}{764}$

i.e. leucopenia

(3) 2 p.m. *temperature* 38.2 — $\frac{WC}{RC} = \frac{1}{968}$

i.e., increased leucopenia

The leucocytosis was in this case quite transient followed by a marked leucopenia

During the course of an attack we may have changes of this kind —

1 Some days before the attack and before parasites appear in the blood instead of

$\frac{WC}{RC} = \frac{1}{500}$ $\frac{WC}{RC} = \frac{1}{1000}$ i e a leucopenia

2 During the shivering attack and height of the pyrexia the condition changes to one of leucocytosis so that

$\frac{WC}{RC} = \frac{1}{300}$ $\frac{1}{200}$ or even $\frac{1}{90}$

3 This leucocytosis may not last long but is followed again by a marked *leucopenia* which is at its maximum before the onset of the next attack

$\frac{WC}{RC}$ instead of $\frac{1}{500}$ may be $\frac{1}{800}$

BILLET (Fig 6) who has traced out hourly the relation of the leucocyte curve to the temperature curve has shewn that in regular curves of the tertian or quartan type the leucocytic curve follows closely the variations in the temperature Thus before the febrile attack in a quartan there may be a leucopenia represented by $\frac{WC}{RC} = \frac{1}{1200}$ at the time of the attack however there is a leucocytosis of $\frac{WC}{RC} = \frac{1}{200}$

This gradually disappears passing through the normal value $\frac{1}{500}$ and again reaching a marked *leucopenia* before the next attack The variations are of the same kind in irregular

temperitures, the leucocytosis corresponding to the rise of temperiture, and the leucopenia to the apyretic intervals

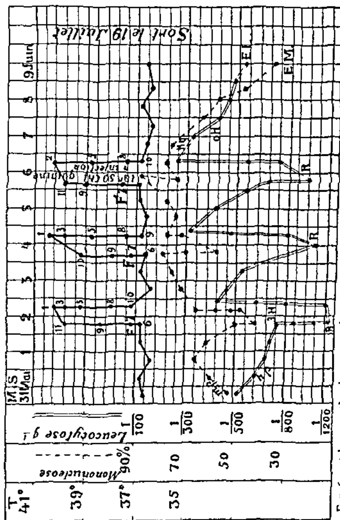


Fig (Showing th changes in the total Leucccytes (and in the percentage of mononuclears large and small) in a case of simple tertian fever. The double line curve = that of total Leucocytes (After BILLET)

The Percentage Value of the Leucocytes.—If we now make a differential count in a stained specimen we shall be able to ascertain what change if any there is in the relative percentage of the different kinds (for normal values *vide* p 42)

1 The main *characteristic* change is that there is an increase in the percentage of large mononuclears so that at times they may even outnumber the polynuclear

2 The change is especially well marked in the periods of apyrexia (*i e* when there is a leucopenia) When there is a leucocytosis the increase in the mononuclears may not be apparent

As examples of this leucocytic change we may give the following —

(i) Small mononuclear	18 1 per cent
Large mononuclear and transitional	31 4
Polynuclear	50 [illegible]
Eosinophil	0 4

A fatal case of malignant tertian (BASTIANELLI)

(ii) Small mononuclear	19 1 per cent
Large mononuclear and transitional	41 0
Polynuclear	39 0
Eosinophil	0 6

A fatal case of comatose malignant tertian (BASTIANELLI)

PANSE —Malignant tertian fever t [illegible]7 [illegible] C

(iii) Small mononuclear	18 1 per cent
Large mononuclear and transitional	[illegible]6 4
Polynuclear	55 3

Z f J Hyg 9 3 p 59

t 976 F Malignant tertian

(iv)	Small mononuclear	14 8 per cent
	Large mononuclear and transitional	46 7 "
	Polynuclear	38 5 "

The figures are by no means always as high as this but as we have already said (p 41) we consider a value above fifteen per cent as diagnostic of malaria The higher values are appreciated at once by an inspection of the slide where the large mononuclears seem to occur in every field and may be pigmented For the low values a careful count is required

An increase in the large mononuclears has been found in one case of human trypanosomiasis This has not so far been confirmed, but if it is it can but slightly effect the value of the counts in malaria as a diagnostic means for the clinical features of trypanosomiasis so far as known are extremely characteristic and the chance of an *European* being infected with the disease does not appear to be great the two known cases having occurred in tropical Africa Further, together with the increase of the mononuclears in malaria there are if thorough search is made also pigmented leucocytes to be found The *relative* count of malaria is of great assistance in at least two conditions (1) in those cases where quinine has been taken (2) where consequently the diagnosis is uncertain and the question of typhoid fever arises As we shall now see the *relative* count in typhoid is quite different from that of malaria

Typhoid Fever

During the first week (of uncomplicated cases) the leucocytes are normal.

During the second week there is a *leucopenia*, *e.g.* 2,000, and the leucopenia is in proportion to the severity of the disease.

During the third and fourth weeks the *leucopenia* is still more marked, though also a leucocytosis may be found without any apparent cause.

Relative Leucocyte Values.—During the third, fourth and fifth weeks the mononuclears, *large and small*, may reach the values of forty to sixty per cent., and among these the proportion of *small* mononuclears is very striking.

Pneumonia

There is very early a leucocytosis, *e.g.* 25,000, four hours after the initial chill. The maximum occurs as a rule just before the crisis. The number may fall from a high value to normal in twenty-four hours. Leucocytosis is said to bear a relation to the amount of exudation (*i.e.* lobes involved).

Relative count—

Large and small mononuclear	2 to 4 per cent.
Polynuclear	90 to 95
Eosinophil	rare

The Widal Reaction in Typhoid

While we consider it is not going too far to say that typhoid and malaria can be readily distinguished by the leucocytic count, yet seeing that

in the WIDAL reaction we have an easy means of diagnosing typhoid, the application of this test is of the greatest service in those cases where the diagnosis of malaria or typhoid remains doubtful We shall describe briefly how the test is carried out There is no necessity for specially constructed bulbs or graduated pipettes as often thought

1 Draw out a piece of glass tubing so as to make a pipette having a fine end about the diameter of a hypodermic needle

2 Collect enough blood to fill the pipette to the height of about half an inch The blood will readily flow in if the pipette is held sloping downwards Seal off the fine end in a flame Centrifugalize, if convenient but abundance of serum can be got without by allowing to clot

3 *To Dilute the Serum*—Draw out a piece of glass tubing into a long fine filament take a piece about six inches long make an ink mark about half an inch from the end of the tube insert *this marked end into the tube containing* serum (and clot) and allow serum to flow up to the ink mark then let a distinct bubble of air follow (the size of this bubble does not signify) next allow broth to flow up to ink mark repeat this procedure until nine drops of broth are in the tube these are now each separated by an air bubble and also by a bubble from the serum The dilution is now one in ten

Blow out all the drop on to a slide or watch glass and mix by sucking up and blowing out a few times

4 Take a drop of the diluted serum in a fresh piece of tubing make a mark as before and then allow broth to flow up this gives a dilution

of one in twenty a second drop one in thirty third drop one in forty and finally a drop of typhoid emulsion thus gives a dilution of serum of one in fifty containing typhoid bacilli The whole process of dilution takes less than five minutes

5 *Typhoid Emulsion* — A bacillus should be used that is known to be active Take a fresh over night agar culture and make a fairly thick emulsion in broth or salt solution

6 *Dilution and Time Reaction* — A dilution of one in fifty with a time limit of half an hour may be used With a less diluted serum the time limit must be less

7 Whatever time limit and dilution be used it is very necessary to perform controls from time to time with a variety of other cases to make sure that the agglutination if produced is not produced by normal sera

The Isotonic Point of Tonicity of the Blood

If a drop of blood is allowed to drop into a one per cent solution of salt in a small test tube and stirred up the uniformly turbid solution will eventually become clear when the corpuscles have settled at the bottom and the supernatant fluid will be unchanged if on the contrary we add another drop of blood to a little water in a test tube the whole drop is immediately *laked* and we have resulting a solution of haemoglobin The former solution of salt is called hypertonic the latter solution of water hypotonic Now if we start with such a hypertonic solution one per cent salt and proceed gradually to dilute it we shall

eventually reach a strength where the hypotonic, *i.e.*, hæmolysing effect begins to appear. The strength of salt solution just above this where no change occurs is the isotonic point for the particular blood in question. This point then gives us information as to the resistance to a hæmolytic action of the corpuscles. The blood in various diseases is found to vary in regard to the strength of salt required to prevent hæmolysis. So that if a normal blood is unchanged by a 0.5 per cent. salt solution, whereas an abnormal requires 0.6 per cent. to protect it, the latter blood is described as having a *less* resistance than the former, but it has a *higher* isotonic point.

The determination of the isotonic point then gives us a more definite notion of the state of the blood in disease than does a mere determination of the hæmoglobin. The isotonic point of human blood is about 0.41 per cent. salt solution.

To Determine the Isotonic Point.

1. Measure out one c.c. of each salt solution of descending strengths, 0.43 per cent., 0.41 per cent., 0.39 per cent., etc., into four small test tubes and one c.c. of water into a fifth tube.
2. Add to each the amount of blood contained in two divisions of the stem of a Thoma-Zeiss pipette (the whole stem contains ten divisions).
3. Allow to stand for some time. Some of the solutions will have hæmoglobin in solution.
4. The amount of hæmoglobin in each tube can be estimated by adding the amount of a normal blood in two divisions to one c.c. of water. Call

this = 100 per cent Dilute this solution so that a number of tubes equal to ninety eighty, seventy etc per cent are got Compare the tubes containing the salt solutions directly with these

In malaria the resistance of the blood is markedly lowered thus whereas in a control normal blood a 0.41 per cent salt solution gave no haemolysis in the case of two malaria patients the haemolysis was equal to twenty five per cent and forty per cent respectively

In blackwater fever on the contrary a raised resistance of the blood may be found

Clinical Study of Malaria

The Urine While not proposing here to consider the general reactions of the urine in malaria for which we must refer the reader to any standard text book yet we think it useful to consider some points which are of more particular interest It is especially in blackwater that we still require complete analyses of the urine and more especially in those who are constantly subject to malarial attacks and are at the same time taking quinine It is possible that such analyses might give us indications which would enable us to avert the danger of an attack of blackwater fever and to determine when quinine should not be given We have not considered here the method of examining the urine by cryoscopy as it is not at present a practical clinical method but its possibilities should not be forgotten

Albuminuria —The occurrence of albuminuria in malaria varies according to the particular country thus in Rome it is uncommon in Senegal,

on the contrary exceedingly common This is an illustration of the often neglected fact that tropical malaria differs in many ways from malaria of temperate climes

Filter the urine if morphological constituents are present as is the case in blackwater fever through two thicknesses of filter paper or add some calcined magnesia then filter Place some urine in a urine glass and with a pipette reaching to the bottom allow half as much nitric acid to slowly trickle in (SIMON) A white cloud at the junction layer indicates *serum albumin* (globulin or peptones) Urea nitrate crystals will often separate out at this junction layer

Serum Globulin —Make the urine alkaline with ammonia *filter off any precipitated phosphates* to the urine add an equal volume of saturated solution of ammonia sulphate A precipitate indicates globulins or the formation of the precipitate may be seen at the junction layer Test filtrate for albumin by adding excess of acetic acid and boiling

Albumoses —Acidify the urine with acetic acid add an equal volume of a saturated solution of salt boil if a precipitate occurs (albumen) filter hot Albumoses separate out on *cooling* or to the hot filtrate add caustic soda solution then dilute copper solution gradually a red colour signifies albumoses

NOTE —Urines rich in urobilin (*e g* malaria and blackwater fever) will give this biuret reaction

In presence of urobilin to ten c c of urine add eight grammes of powdered ammonium sulphate until dissolved boil for a few seconds the albumoses are precipitated on the sides of the

test tube pour off the urine and wash the pre cipitate with alcohol then chloroform dissolve in water and apply the biuret test Test the alcoholic extract for urobilin

Nucleo Albumens —Filter the urine carefully boil to remove albumen then add gradually excess of strong acetic acid A turbidity indicates nucleo albumen

Blood (Haemoglobin etc)

1 *Examine Spectroscopically* (Fig 63) —If the bands of methaemoglobin or oxyhaemoglobin are seen confirm by adding ammonium sulphide when the bands of reduced haemoglobin are got

2 *Heller's Test* —Make the urine strongly alkaline with caustic soda boil the precipitate in the presence of haemoglobin is bright red confirm by dissolving the filtered precipitate in acetic acid a red solution is formed (spectroscopically this gives the characteristic bands of haemachromogen)

3 *Guaiacum Test* — Equal parts of tincture of guaiacum and oil of turpentine (which has been exposed to the air) are taken add slowly to the urine A blue ring is formed at the junction layer

Methaemoglobin

The urine in blackwater fever when examined early most frequently contains blood pigment in this form later oxyhaemoglobin This according to Hoppe Seyler also holds good for every urine with haemoglobin in solution

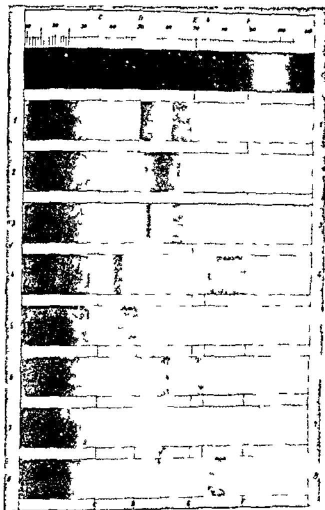


Fig. 63. Spectra. (1) Oxyhaemoglobin (2) Haemoglobin (3) Alkaline Methaemoglobin (4) Methaemoglobin in Neutral or Acid Solution (5) Alkaline Haematin (6) Reduced Haematin Haemochromogen (7) Urobilin (8) Zinc Urobilin

The Characters of Methaemoglobin are —

In *acid* solution the oxyhaemoglobin bands are weak or invisible There is a band *between* C and D nearer the former The band of acid haematin is similar in position It is however close to C

In *alkaline* solution the acid band disappears and a faint band on the red side of D takes its place (compare with alkaline haematin)

Reduced by ammonium sulphide the bands of reduced haemoglobin are got It differs from oxyhaemoglobin in its chemical reactions by the fact that it is precipitated by basic or neutral lead acetate solution whereas oxyhaemoglobin is not

Detection —

1 In presence of oxyhaemoglobin Ppt with basic lead acetate filter decompose the precipitate with carbonate of soda solution examine for the bands of alkaline methaemoglobin

2 In presence of urobilin Proceed in the same way

3 In presence of bile pigment Precipitate these by making the solution alkaline with ammonia after adding CaCl

4 In neutral solutions its spectrum is identical with that of haematin in natural solutions (NEUBAULT and VOGEL) Reduced by $(NH_4)S$ methaemoglobin is changed to reduced haemoglobin and haematin to reduced haematin the bands of which are easily recognized

UROBILIN

Frequently occurs in the urine in jaundice instead of bile pigment

According to HAYEM it is associated with methaemoglobinaemia. Its occurrence in black water fever is very common, occasionally before the attacks, but more constantly after the oxy-haemoglobin has disappeared or together with it.

Characteristics.—

1. In *acid* urine a band near F occurs, between 88 and 101.

2. In *alkaline* urine a band between 81 and 95.

3. Make the urine strongly alkaline with ammonia, filter, add $ZnCl_2$ solution but not sufficient to form a permanent precipitate.

A green fluorescence occurs and the much clearer band nearer 'b' than the acid band.

Detection.—

1. *If oxyhaemoglobin is present*. *Precipitate* the urobilin with *basic* lead acetate, then acidify the precipitate, when the urobilin goes into solution.

2. If methaemoglobin is present. *Neutralize* the urine with carbonate of soda, precipitate the methaemoglobin with neutral lead acetate. Filter, test the filtrate for urobilin.

BILE PIGMENTS

Where urobilin is present, as in blackwater, the colour of the foam on shaking the urine, the staining of the filter paper etc. cannot be regarded as satisfactory tests.

Detection.—

1. *Gmelin Rosenbach Test*.—Filter the urine through filter paper (Swedish). Dry, apply a drop of nitric acid (fuming) to this, a play of colours is got.

2 *Huppert's Test*—Precipitate the urine with $BaCl_2$. Filter, wash the residue off the filter (perforated) with acidulated H_2SO_4 alcohol. Boil. A bright green colour indicates bilirubin.

3 *Smith's Test*—To ten c.c. of the urine add two c.c. of dilute tincture of iodine (tincture of iodine 1, alcohol 10). A green ring forms at the junction zone.

Bilirubin and Haematoidin (in Urinary Sediment)

1 Bilirubin crystals form yellowish brown rhomboidal plates or needles.

Easily soluble in $CHCl_3$. Gives Gmelin's reaction green under the microscope.

2 Haematoidin, dark red in colour or greenish if impure; with nitric acid they give a transient blue.

According to Hoppe-Seyler, however, they are identical.

Haematoporphyrin

Occurs in urine as alkaline haematoporphyrin (Fig. 64). In urate sediments a similar form occurs. It is soluble in chloroform giving bands similar to those of oxyhaemoglobin, but acid converts this into acid haematoporphyrin bands. Solutions have a brilliant red fluorescence. It is found in the urine in toxic conditions such as chronic sulphonal poisoning. It is precipitated by lead acetate while oxyhaemoglobin is not.

Sugar

Before testing for sugar, boil to remove all proteids.

Reduction of copper solution is effected by bile pigments Reduction occurs also in patients taking salicylic acid, sulphonal, and quinine (SIMON), so that it may be necessary to use—

1 *Fermentation Test* or

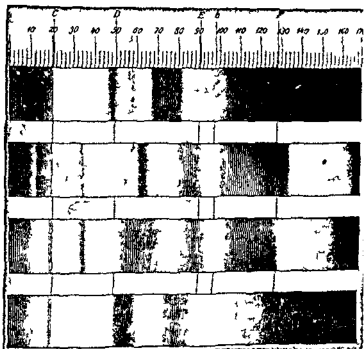


Fig 64 *Spectra of* (1) *Haematoporphyrin* (*in Acid Solution*) (2) *Haematoporphyrin in Alkaline Solution* (3) *in Neutral Solution* (4) *Haematoporphyrin* (*urate sediments*)

2 *Phenyl Hydrazine Test* —Take five drops of pure phenyl hydrazine ten drops glacial acetic acid, one c c of a saturated solution of common

salt add three c.c. of urine boil for three minutes cool crystals separate out in a few minutes up to one hour This is an exceedingly delicate test

THE DETECTION OF QUININE IN THE URINE *

The detection of quinine in the urine is of importance in connexion with the property that this drug has of inducing attacks of haemoglobinuria (blackwater fever) in patients resident in regions where malaria is especially virulent, and where generally the parasite form is the malignant tertian associated with an extremely high endemic index of native (children) malaria

Two hundred c.c. of urine are acidified with some drops of sulphuric acid A spoonful of solid picric acid is then added The solution is allowed to stand for an hour and then filtered The solution should be quite clear and should give with a saturated solution of picric acid no turbidity If there is difficulty in getting a clear filtrate add a trace of egg albumen and filter again The half dry residue is then digested in an Erlenmeyer flask with fifty c.c. of 30 per cent soda solution for half an hour on the water bath Now add sixty c.c. chloroform shake for two hours in a shaking apparatus The solution of chloroform is now removed by means of a separating funnel and collected in a weighed flask The flask should have a long neck to prevent spurting Evaporate in a water bath and dry at 120° C The residue is quinine The experimental error is only one to two per cent

* Kl Z f f Hyg Bd H 3 460

Determination of the Periodicity of Parasite Development

The inspection of a temperature chart is not in itself sufficient to determine the cycle of development of a parasite Thus as is well known a quotidian temperature chart may be produced by a double tertian (simple) or by a triple quartan infection If then in the case of the double tertian we made microscopical examinations at definite intervals for forty eight hours, we should find in the blood at any particular time parasites in *two* phases of development corresponding to each cycle The accompanying chart shews how in the case of what proved to be the malignant tertian parasite we were able to establish the cycle of development We proceeded to make blood examinations at frequent intervals (four hours) We found that at any particular time parasites of various sizes might be found but by counting several hundred parasites in each film and estimating their size with a micrometer we found that at any particular time there was a preponderance of parasites of one size Thus at ten p m on the 2nd there are numerous small forms *i e* about one seventh to one eighth of a red cell in diameter and it is not till ten p m (about) on the 4th that the same condition of blood is found again accordingly the parasite had a developmental cycle of forty eight hours (approximately) And further we determined the periods taken to develop from small forms to largest forms in the peripheral blood (about eighteen hours) and the disappearance of these and the reappearance of numerous youngest parasites (about thirty hours)

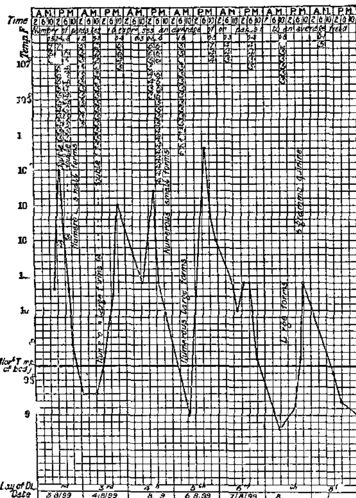


Fig 65 Illustrati m th d of determing th d lopment l Cycl of a P rasite (Th fi ur ithin the l r pr ent th size of th I arasites—thus 7 signifies the P ra te is one seventh the diameter of a red ell)

So that by determing in these three periods we were able to conclude that the parasite was the malignant tertian

In order then to determine the cycle of a parasite it is necessary —

1 To estimate the size and percentage of parasites of each size at any particular time e g starting with the onset of the attack

2 To follow each group to its period of maximum development in the circulation

3 To estimate the time between this period and the next appearance of young forms

4 To estimate the time between the appearance of an outburst of young forms (No 1) and a second similar outburst (No 4)

The interval between one and four should be equal to the sum of the intervals of periods two and three It is more accurate to use a micrometer scale for measuring but the estimation can be made with considerable accuracy without

If we are dealing with three generations of parasites as in a triple quartan the principle is precisely the same though it may require careful observation to separate the different groups though in this particular case the process is facilitated by the presence of segmenting and pre segmenting bodies which are easily counted In order then to establish a parasite cycle repeated observations at definite intervals are necessary, and also the temperature should be carefully recorded every four hours or two hours as considerable variations may otherwise escape observation

Simple Tertian

Examined during the commencement of apyrexia young parasites are found one fifth to one ninth the size of the red cell The corpuscles may be slightly larger than normal On the following day the parasites occupy one half to two thirds of the corpuscle much pigmented but not so actively motile as the smaller ones The red cells are much enlarged at the end of the second day presegmenting forms are found Division into four to six or more parts may take place six hours before an attack but true fission forms are only found two to three hours before an attack These forms have sixteen to twenty spores so that here again the multiplication of a group of parasites may be shown microscopically to coincide with a febrile attack But as opposed to quartan the actual number of fission forms in the peripheral circulation is small they are far more numerous in the spleen

Double Tertian

The interval between the development of the two groups of parasites is about twenty four hours So that if a blood examination be made just before an attack fission forms will be found and parasites about half grown Here again by following out the development periodically these latter forms will be found to sporulate on the next day

Quartan Parasite

If the blood be examined as soon as apyrexia sets in, the corpuscles will contain young parasites

one fifth to one sixth as large as the red cells growth is progressive during the apyrexia, and six to ten hours before the next attack 'presegmenting' forms will be found. The parasite now fills the cell which is not enlarged. The pigment is arranged in radiating bands. The next stage is the concentration of the pigment in a central mass and it is seen in fresh or stained specimens that the cytoplasm is divided into eight to ten segments or oval bodies (daisy form). This segmentation is nearly coincident with the next attack, but parasites in complete fission may be found five to six hours earlier. As the temperature rises these sporulating forms disappear and again young forms are found in the red cells. The quartan then goes through all its stages in the peripheral circulation.

Double Quartan

Two groups of parasites going through the above regular cycle with about a day's interval may be followed in the blood.

Triple Quartan

There are three distinct groups sporulating on successive days.

Malignant Tertian (Tropical)

During the pyrexia small forms one eighth [one fifth] of the red cell may be found. *They persist during the pyrexia, i.e.* for the greater part of a day. The parasites at this stage may be extraordinarily few.

During the apyrexia forms one fourth to one third of a red cell are found and the parasites are found in greatest numbers Fission and presegmenting forms are extremely rare in the tropics During the next attack the young forms are again found and so the time of the cycle as we have shown above may be deduced and may be controlled by observation of intermediate stages

QUOTIDIAN

Parasites have been described which complete their development in twenty four hours (about) Thus at the pyrexia young forms occur During the apyretic interval large forms and presegmenting forms and again at the next attack young forms thus developing in twenty four hours As we have stated above to establish accurately this cycle three periods would have to be traced —

No 1 (? Twelve hours) from young forms to largest forms

No 2 (? Twelve hours) from largest forms to young forms

No 3 Twenty four hours from young forms to young forms

While some consider that the quotidian temperature is due to the fact that the malignant tertian has a very variable period of development viz twenty four to forty eight hours and in fact all intermediate times others consider that with one generation of parasites there is a second accumulation of young forms in sufficient quantity to produce a quotidian attack

In quotidian fever due to the malignant tertian parasite the characteristic febrile attack

with its preliminary pseudo crisis is lost attack instead of lasting about a day lasts a hours only, as in the simple tertian, and inst of a pseudo crisis there is a true crisis, but young parasites, as in the malignant tertian att are still coming into the circulation and th follows a rise which replaces the apyretic day the ordinary malignant tertian

According to MAURER, in the case of quotidian chart produced *by one generation malignant tertian parasites* we have the feb attack produced by the division of the major of the segmenting forms and then a fall to norm occurs when, however there is a suffici *accumulation of young forms arising from* same generation there is again a rise giving quotidian chart As we have seen, the you forms of the malignant tertian parasite pers during the pyrexia If however by any me they are destroyed or cease appearing temporar during the day of pyrexia we should get a fall *normal* and then as soon as this inhibiting ca was removed again a rise giving a quotidian ch produced by the malignant tertian parasite

IRREGULAR TEMPERATURES

Besides the typical malignant tertian ter perature chart and the quotidian chart, vario irregular temperatures may occur, due solely the malignant tertian parasite Such charts *not at all uncommon* in first attacks in the tropi and may be followed by charts with regul curves

The malignant tertian parasite has a develo mental cycle of about forty eight hours, and it seem

more likely that these irregular charts are produced by an irregular irruption of young forms into the circulation than that the parasite has a variable time of development. If we suppose that young fission forms exist in the internal organs but do not commence their growth in the circulating red cells, but come into the circulation irregularly, then we should have still a constant time of development but an inconstant time at which the development started. If however a quotidian parasite exists there should be no difficulty, as we have stated above, in determining the fact by a series of measurements at fixed intervals.

ACTION OF QUININE

The data of different investigators into the absorption and elimination of quinine exhibit considerable differences dependent upon the different conditions of experiment and the mode of estimation employed. The following statements must therefore be received with caution:—

1. *According to KERNER the elimination of*—

Quinine hydrochlorate begins in fifteen minutes and ends in forty-eight hours.

Sulphate (neutral) begins in thirty minutes and ends in forty-eight hours.

Sulphate (basic) begins in forty-five minutes and ends in sixty hours.

2. *Mode of Administration.*—

Per os quinine appears in the urine in thirty to fifty minutes.

Per rectum quinine appears in the urine in eighteen to twenty minutes.

Subcutaneously quinine appears in the urine in twelve to twenty minutes

3 *Duration of Elimination*—

According to Garofalo it lasts one and a half to seven and three quarter hours

According to Dietl it lasts forty eight hours

According to Byasson it lasts seventy two hours

According to Personni it lasts eight days

4 *The Acme of Elimination*—

According to Thau and Kerner after the first six hours

According to Garofalo after the first one and a half to four hours

According to Kleine after the first three to six hours

5 *Hypodermic Injection*—Garofalo states that the elimination is rapid and that larger doses can be accumulated in the blood in a shorter time by this method than by doses given by the mouth while Kleine states that the absorption by this method is slow Kleine's figures will be given below

6 *Amount of Quinine Eliminated*—

Welitschowski	100 per cent about	
Kerner	95	
Byasson	75	,
Kleine	9 27	, (*vide* later)
Personne	16	,
Merkel	13	
Mariani during first day	18 7 per cent	
second ,	6 3	
, third	1 3	,
, fourth	0 7	,
Total	27 per cent	

7 KLEINE s data as to the amount eliminated in twenty four hours —

Per os Administration—

(i) 25 34 per cent
(ii) 19 71
(iii) 27 29
(iv) 9 67

This low value No 4 is explained by the fact that the quinine was given on a full stomach whereas in the three other results the quinine had been given to the patient fasting

Per Clysma— (i) 17 66 per cent
(ii) 17 15
(iii) 17 84

Subcutaneously—(i) 11 37
(ii) 9 70
(iii) 15 32

Now although proportionately a smaller amount is excreted in this way (and this is possibly in conformity with the clinical experience that ringing in the ears and other unpleasant symptoms of quinine are generally absent after subcutaneous injection) yet it is probable that the excretion is a more prolonged one than by the other methods for deposits of quinine can still be found at the site of injection some weeks later and so the undoubted efficacy of this mode of treament may really be due to its prolonged action (and elimination)

MARIANI s results also shew that after an injection of quinine into the muscles of a rabbit about twenty four hours later half the amount could still be extracted from the muscles KLEINE and MARIANI s results shew that a full stomach inhibits markedly the absorption of quinine so also any catarrhal state is prejudicial

two to three frequently, five to six or possibly twenty four hours

2 The amount of quinine does not determine whether the haemoglobinuria is slight or severe

3 After haemoglobinuria has been produced by quinine a second administration does not necessarily produce a second attack of haemoglobinuria

These facts clearly shew that it is not the quinine *per se* but a condition of blood in the particular malarial patient which is the determining factor whether quinine will produce an attack

This is further borne out by the well known fact that the aborigines rarely if ever, suffer from haemoglobinuria but it is in Europeans subjected to unnatural climatic conditions and subjected to virulent malaria that the disease is most frequently found

We would only add finally, that it is quite illogical to abstain from quinine in malaria, on the contrary its *adequate* administration would prevent the occurrence of these attacks

As we have already said, an accurate study of the urine in these cases and in allied cases of malaria where quinine produces urobilinuria is necessary

Especially important is the study of the urine and the blood in the prehaemoglobinuric state It would of course involve an accurate study of all possible subjects of the disease and more especially those who had already had an attack

POST MORTEM CHANGES IN MALARIA (MARCHIAFAVA AND BIGNAMI)

Brain —

1 Punctiform haemorrhages of the meninges

2 Punctiform haemorrhages of the white substance of the brain

3 The brain capillaries may contain nearly every red cell infected Sporulating forms are especially common



Fig 66 Showing deposition of Pigment in Liver (left) Spleen (right) and Sporulating Parasites in Brain Capillary (bottom)

4 The capillary endothelium may show fatty degeneration together with pigmentation and sometimes parasites

5 Similar appearances are also found in the vessel of the pia mater

Lungs —

1 Large pigmented mononuclears in the capillaries but especially in the veins In the lungs especially phagocytosis is proceeding

2 There is a terminal infection with the diplococcus pneumoniae

Spleen — The trabeculae of the pulp are distended by infected red cells and pigmented large mononuclears are abundant. The malpighian follicles on the contrary are non pigmented.

Liver Endothelium of capillaries is swollen and pigmented. Pigment is also found in Kupffer's cells. The liver cells contain only haemosiderin not melanin. Pigmentation is most intense around the central veins.

Kidneys —Pigmentation is much less marked. Changes may occur in the epithelium of the tubules independent of the presence of parasites.

Bone Marrow—Parasites and melanin free, and in large mononuclear, leucocytes and microphages are found. Crescents may be found here when absent or scanty elsewhere as in the spleen and brain; it is consequently supposed that they principally develop here.

In cases of malaria of long standing the yellow marrow becomes red.

Stomach and Intestines—In malaria with choleraic or haemorrhagic symptoms parasites may abound in the capillaries of the villi.

Chronic Malaria

Spleen — As is well known the spleen may in these cases fill the whole abdomen. Dilatation of the various lacunae occurs with a thickening of the splenic reticulum. The pigment tends to become deposited eventually in the connective tissue surrounding the follicles. The splenic septa become thickened.

Liver—The pigment is found mainly in the *periphery* of the lobules and pigment in the form of blocks in the perivascular connective tissue

The capillaries are much dilated and the epithelium contains blocks of pigment Atrophy of the liver cells and their nuclei occurs

Bone Marrow—The marrow of the long bones is usually red due to a large development of haematoblastic tissue Normoblasts are common

Pigment disappears rapidly from the bone marrow

LITERATURE

Marchiafava and Bignami *Twentieth Century Practice of Medicine* Malaria Vol XIX S Low Marston and Co This comprehensive and learned treatise is incomparably the best in the English language dealing with all aspects of malaria and also blackwater fever

Chapter XXI

BLACKWATER FEVER

Microscopical investigations in this disease are frequently negative as regards malarial parasites but it is all important when the examination is made, as the following analysis of over one hundred cases microscopically examined shows —

Parasites are present the day *before* the attack in ninety five per cent of cases

Parasites are present the day *of* the attack in seventy per cent of cases

Parasites are present the day *after* the attack in twenty per cent of cases

In a series of cases examined by ourselves in British Central Africa we found malarial parasites only in 12.5 per cent but as we have already shown we have two further tests for a malarial infection —

(1) The increase in the percentage of large mononuclear leucocytes

(2) The presence of pigmented large mononuclear leucocytes

By using these tests we were able to prove that 93.7 per cent not 12.5 per cent of our cases were due to a malarial infection

Further in the only case of blackwater fever seen by us *before* the onset of haemoglobinuria *parasites were present* in abundance, afterwards they rapidly disappeared

For the details of the proof we must refer to the original papers where also the causative action of quinine is discussed That quinine is the factor which in the large majority of cases determines the onset of haemoglobinuria appears to us equally certain

EXAMINATION OF THE BLOOD IN BLACKWATER FEVER

1 Note the difficulty in obtaining a full sized drop of blood

2 Observe the thin nature of the blood drop its oily nature and the difficulty with which it adheres to the slide These properties are best seen in severe cases

3 Collect a specimen in a fine pipette and allow the serum to separate Observe whether the serum is yellow (cholaemia) or reddish (haemoglobinuria) using the spectroscope if necessary

4 To some of patient's serum add normal blood Observe whether there is any haemolysis (using a haemocytometer if necessary)

5 Determine tonicity of patient's blood Rate of coagulation approximately by placing several drops on a glass slide

6 Count the red and white cells The red cells are as a rule quite normal in shape

7 Determine the amount of haemoglobin

8 Make films every two hours if possible (*as early as possible*) noting accurately the time and temperature at which the films are made

9 Examine films for parasites if these are absent search carefully several large films for

pigmented leucocytes, as these, as also in ordinary malaria, may require long search

10 Make careful differential counts of the leucocytes, especially when the temperature is falling, as it is then that the mononuclear increase is most marked When the temperature is raised (e g, 103 to 105) the polynuclears may reach ninety per cent

11 *Observe presence of normoblasts meg alo blasts* various abnormal staining reactions, *e g*, polychromatophilia of the red cell, especially during recovery

12 Make careful blood counts immediately before and after administering quinine when no haemoglobinuria results According to PANSE* there may result a blood destruction due to the quinine which does not shew itself as haemoglobinuria

EXAMINATION OF THE URINE IN BLACKWATER FEVER

1 Before the attack (if possible) examine for albumen, urobilin reducing bodies, etc

2 Examine so called high coloured urines As a rule these do not shew bile pigment

3 Examine urine during an attack for methaemoglobin (or haematin) oxyhaemoglobin, urobilin bile pigment bilirubin crystals, hae globin casts granular or hyaline casts blood cells, etc

4 Centrifugalize the urine Examine the clear layer (as in 3), and make films of the sediment

* P nse *Z n k f t f Hyg* 1903 1

The sediment may contain hyaline and granular casts stained with haemoglobin The mass of the sediment however, consists of masses of haemoglobin of a yellowish red colour

Post Mortem Examination

1 Make smear preparations of spleen kidney liver bone marrow brain etc Examine for parasites and pigmented leucocytes Parasites are generally absent but pigmented leucocytes may occur in large number in the spleen Fine pigment is also found in the liver in endothelial capillary cells (Fig 66)

2 Cut sections especially of brain tissue as parasites may be found here and nowhere else

Urobilinuria

As we have indicated elsewhere the occurrence of urobilin may be an important indication in cases where a susceptibility to quinine haemoglobinuria exists thus in Murri's case a girl had haemoglobinuria eight times between August 3 1894 and April 6 1895 following upon the administration eight times of small doses of quinine From 1895 to 1897 the girl remained well On March 27 1897 she was given 0 5 grammes of quinine to see whether her disposition to quinine poisoning still remained The result was fever vomiting of bile, etc albuminuria peptonuria and urobilinuria (not haemoglobinuria)

A Plehn in a recent paper points out a peculiar property of the urine sometimes observed in blackwater cases On boiling the

urine and allowing to stand for some time, a bright purple colour appears

We have observed that blackwater urines made alkaline with potash, and then boiled produce a purple colour, giving the bands of haemochromogen (reduced haematin), shewing that the urine itself contained reducing bodies

Whether PLEHN's purple colour is the same we cannot say

THE KIDNEYS IN BLACKWATER

A PLEHN, in the same paper, argues that

1 There is not usually nephritis in blackwater (although this may exist as a complication)

2 Oedema is absent

3 Cylinders are seldom present in the urine, red cell and leucocytes in uncomplicated cases are also absent

4 Amorphous black red pigment only in severest cases What PLEHN means by this is not clear The remains of haemoglobinurous stromata seen in severe cases, are stained a deep yellow

5 Bile pigment is rare, if present there is generally true nephritis

6 According to PLEHN, there is little alteration of the tubular epithelium, in our experience however this may be intense

7 Sometimes insignificant sclerosis and small cell infiltration (but this depends on malaria)

8 In blackwater there is a *functional* disturbance of the kidneys (*cp* paroxysmal haemoglobinuria)

9 During anuria no symptoms of renal colic as there would be if there were a blocking

10 Absence of all uraemic symptoms proves that cessation of function of kidneys does not necessarily lead to uraemic symptoms

LITERATURE

KOCH *Zeitschrift für Hygiene* (Bd XXX 1899 p 95)
STEPHENS and CHRISTOPHERS *Reports to the Malarial Committee of the Royal Society* Harrison & Son London
LANSE *Zeitschrift für Hygiene* (1903 s 1)
Twentieth Century Dictionary of Medicine *Malaria* The fullest and best account in the English language

urine and allowing to stand for some time, a bright purple colour appears

We have observed that black water urines made alkaline with potash, and then boiled produce a purple colour, giving the bands of haemochromogen (reduced haematin) shewing that the urine itself contained reducing bodies

Whether PLEHN's purple colour is the same we cannot say

THE KIDNEYS IN BLACKWATER

A PLEHN, in the same paper, argues that

1 There is not usually nephritis in black water (although this may exist as a complication)

2 Oedema is absent

3 Cylinders are seldom present in the urine, red cell and leucocytes in uncomplicated cases are also absent

4 Amorphous 'black red pigment only in severest cases What PLEHN means by this is not clear The remains of haemoglobinurous stromata seen in severe cases, are stained a deep yellow

5 Bile pigment is rare, if present there is generally true nephritis

6 According to PLEHN, there is little alteration of the tubular epithelium, in our experience however this may be intense

7 Sometimes insignificant sclerosis and small cell infiltration (but this depends on malaria)

8 In blackwater there is a *functional* disturbance of the kidneys (*c p* paroxysmal haemoglobinuria)

9 During anuria no symptoms of renal colic as there would be if there were a blocking

10 Absence of all uraemic symptoms proves that cessation of function of kidneys does not necessarily lead to uraemic symptoms

LITERATURE

KOCH *Zeitschrift für Hygiene* (Bd XXX 1899 p 95)
STEPHENS and CHRISTOPHERS *Reports to the Malarial Committee of the Royal Society* Harrison & Son London
PAUSE *Zeitschrift für Hygiene* (1903 s 1)
Twentieth Century Dictionary of Medicine *Malaria* The fullest and best account in the English language

Chapter XXVI

THE HAEMOCYTOZOA—*Continued*

The haemocytozoa, or endoglobular haematozoa are divided by LAVERAN into three genera

1 Genus *Haemamoeba*
2 Genus *Piroplasma*
3 Genus *Haemogregarina*

The genus *haemamoeba* includes the malaria parasites with which we have already fully dealt We now proceed to the other members of the genus, and then to the other two genera which go to make up the endoglobular haematozoa

GENUS HAEMAMOEBA

1 *H Relicta (Proteosoma Grassii)*—Discovered by GRASSI in the blood of birds in Italy In certain regions sparrows and goldfinches are commonly infected Sparrows are frequently infected in India In Africa numerous small birds were examined by us, but proteosoma was never found (only halteridium) Transmission from one bird to another by inoculation is readily effected Canaries are extremely susceptible Pigeons among other birds are immune Birds that have recovered from an infection have acquired a well marked immunity against a subsequent inoculation

The parasite is closely allied to the malaria parasite and is especially suitable for the study of the exogenous mosquito cycle

Endogenous Cycle (Fig 67) —The parasite in its earliest stage is unpigmented Coincident with growth a grain or two of pigment appears and the characteristic property of the parasite shows itself viz the displacement of the nucleus of the red cell so that the nucleus may take up a position at right angles and away from the normal one All stages of development up to segmenting forms are found in the blood at the same time so that no cycle of development can here be followed nor is there any intermission in the clinical symptoms (temperature etc) of infected birds

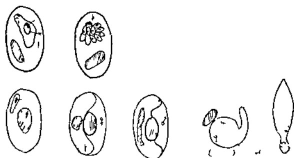


Fig 67 (*Upper line*) *Proteosoma showing medium size Parasite and Segmenting Form* (*Lower line*) *Halteridium young form Female and Male Gametes and Vermicule*

Exogenous Cycle —Besides the asexual sexual forms occur in the blood They are spherical hyaline bodies of two varieties characterized in stained specimens by the same general differences

which distinguish the male and female gametes of the malaria parasite

(i) The male cell possesses a mass of compact chromatin and faintly staining protoplasm

(ii) The female cell possesses but little chromatin but stains deep blue (Romanowsky)

Flagellation —(i) This can be observed in a simple wet film preparation and in hanging drop preparations (p 30), or

(ii) Use artificial serum (bird s serum, one part salt solution, 0 6 per cent, nine parts) and to this add a trace of bird s blood Make a series of hanging drops in moist chambers Dry fix and stain from time to time according to stage of development, observed microscopically

Further stages of development (vermiculi) have not been observed on the slide

Development of Vermiculi —(i) Determine what species of *Culex* is the suitable one for the process of development *C nemorosus* was used by Koch in Italy *C fatigans* is also a carrier

(ii) Collect the *Culex* that have fed on sparrows, etc roosting at night in trees The *Culex* can be caught in large numbers in shaded drains under bridges in outhouses, etc and excellent material is in this way easily got Identify the species of *Culex* that is infected

(iii) For the method of feeding mosquitoes on birds blood *vide* p 101

Twelve to fifteen hours —Vermiculi in all stages of development are found in the stomach a conical projection arises from the fertilized gamete This gradually elongates forming a long, curved, oval body the complete vermiculus The

protoplasm is vacuolated and a nucleus (chromatin) is readily shown by staining (ROMANOWSKY)

The proteosoma vermiculi are larger and more slender than those of halteridium

Development of Zygotes (one to two days) — The vermiculi have disappeared but in the stomach wall are now found transparent spherical pigmented bodies

Three to four days — The zygotes have increased in size and sporoblasts appear in their interior In the larger forms signs of further division are seen (striation) formation of sporozoits

Development of Sporozoits (nine to ten days) — By this time the sporozoits have reached the salivary glands Somewhat earlier they can still be found amidst the thoracic muscle Earlier still they can be pressed out of the ripe oocysts in the stomach wall The sporozoits occupy chiefly the middle lobe of the gland (KOCH)

Black Spores are found in the larger zygotes They also occur free in the thoracic region (or possibly in the gland substance) They are brownish black curved sausage shaped bodies suggesting a mycelial nature It is believed by GRASSI that they are degenerated sporozoits, as they are found within the large sporoblast cysts We have however found them in or about the salivary glands in *Myzomyia Rossii*

2 *H Danilewskyi* (Halteridium) — Occurs almost exclusively in the blood of passerine birds Pigeons are very commonly infected also sparrows finches paddy birds etc

The parasite is characterized by its peculiar curved halter shape embracing the oval nucleus

of the red cell without any displacement of the latter (Fig 67) Young forms are occasionally seen but whether these are young sexual or asexual forms is not determined Segmenting forms and those corresponding to an asexual cycle, as in proteosoma, are unknown

Two varieties of parasites, the male and female gametes, are easily distinguished

(i) Note that the male gamete has a clear hyaline appearance On staining (ROMANOWSKI) a central mass of chromatin is distinguished while the protoplasm is a faint blue Five or more oval pigment grains are placed generally at either extremity

(ii) In fresh specimens the female gamete is finely granular and the pigment is frequently scattered throughout On staining a small amount of chromatin is shewn while the protoplasm takes on a deep blue colour

Flagellation —Select an infected bird that shews numerous gametes in each field Proceed in the same way as in proteosoma The gametes first become spherical and then escape from the red cell The pigment of the male gamete displays violent movement and in a few minutes four to eight flagella are extended The motion of these is at first so rapid that they cannot be distinguished but the corpuscles in the neighbourhood are seen moving In a few minutes one or more breaks off and if fortunately, a female gamete is in the same field the loose flagellum (mikrogamete) can be seen entering the female The pigment of the latter shews active movements at this stage

Vermiculi—The formation can readily be observed on the slide. A conical projection forms at one point of the fertilized gamete (copula). This elongates slowly and gets curved forming an egg shaped or spindle shaped mass. The conical portion eventually separates leaving behind the remains of the cell with the pigment. The vermiculus is thus at first unpigmented but later again it is pigmented (KOCH). In the fresh specimen the protoplasm appears vacuolated and has a nucleus which is readily stained by ROMANOWSKY stain.

Note that the vermiculus (or ookinet) shews forward rotatory and peristaltic motions. The further development of the vermiculi is completely unknown.

Post mortem—Pigment is found in the kidney intestine bone marrow liver and especially the spleen. The brain on the contrary is almost entirely free from it.

It is probable that the halteridia of all birds are not of the same species. Inoculation from one bird to another is extremely difficult if not impossible. This may be due to the fact that the parasites in the blood are in all sexual forms. In monkeys we appear to have a parallel condition viz gametes only in the blood the asexual forms being unknown.

3 *H Kochi*—These haemamoebae occur in monkeys. The forms usually met with are sexual forms. Asexual forms resembling young malarial parasites are very rare. Flagellation can be seen in fresh specimens. The parasites in the fresh film are spherical pale bodies

of the red cell without any displacement of the latter (Fig 67) *Young forms* are occasionally seen but whether these are young sexual or asexual forms is not determined Segmenting forms and those corresponding to an asexual cycle, as in proteosoma are unknown

Two varieties of parasites the male and female gametes are easily distinguished

(i) Note that the male gamete has a clear hyaline appearance On staining (ROMANOWSKI) a central mass of chromatin is distinguished, while the protoplasm is a faint blue Five or more oval pigment grains are placed generally at either extremity

(ii) In fresh specimens the female gamete is finely granular and the pigment is frequently scattered throughout On staining a small amount of chromatin is shewn while the protoplasm takes on a deep blue colour

Flagellation —Select an infected bird that shews numerous gametes in each field Proceed in the same way as in proteosoma The gametes first become spherical and then escape from the red cell The pigment of the male gamete displays violent movement and in a few minutes four to eight flagella are extended The motion of these is at first so rapid that they cannot be distinguished but the corpuscles in the neighbourhood are seen moving In a few minutes one or more breaks off and if fortunately, a female gamete is in the same field, the loose flagellum (mikrogamete) can be seen entering the female The pigment of the latter shews active movements at this stage

Vermiculi.—The formation can readily be observed on the slide. A conical projection forms at one point of the fertilized gamete (copula). This elongates slowly and gets curved forming an egg-shaped or spindle-shaped mass. The conical portion eventually separates leaving behind the remains of the cell with the pigment. The vermiculus is thus at first unpigmented but later again it is pigmented (Koch). In the fresh specimen the protoplasm appears vacuolated and has a nucleus which is readily stained by Romanowsky stain.

Note that the vermiculus (or ookinet) shews forward rotatory and peristaltic motions. The further development of the vermiculi is completely unknown.

Post mortem.—Pigment is found in the kidney, intestine, bone marrow, liver and especially the spleen. The brain on the contrary is almost entirely free from it.

It is probable that the halteridia of all birds are not of the same species. Inoculation from one bird to another is extremely difficult if not impossible. This may be due to the fact that the parasites in the blood are in all sexual forms. In monkeys we appear to have a parallel condition, viz. gametes only in the blood, the asexual forms being unknown.

3. *H. Kochi*.—These haemamoebae occur in monkeys. The forms usually met with are sexual forms. Asexual forms resembling young malaria parasites are very rare. Flagellation can be seen in fresh specimens. The parasites in the fresh film are spherical pale bodies

of the red cell without any displacement of the latter (Fig. 67). Young forms are occasionally seen but whether these are young sexual or asexual forms is not determined. Segmenting forms and those corresponding to an asexual cycle, as in proteosoma are unknown.

Two varieties of parasites, the male and female gametes are easily distinguished.

(i) Note that the *male* gamete has a clear hyaline appearance. On staining (ROMANOWSKY) a central mass of chromatin is distinguished while the protoplasm is a faint blue. Five or more oval pigment grains are placed generally at either extremity.

(ii) In fresh specimens the female gamete is finely granular and the pigment is frequently scattered throughout. On staining a small amount of chromatin is shewn while the protoplasm takes on a deep blue colour.

Flagellation. Select an infected bird that shews numerous gametes in each field. Proceed in the same way as in proteosoma. The gametes first become sphaerical and then escape from the red cell. The pigment of the male gamete displays violent movement and in a few minutes four to eight flagella are extended. The motion of these is at first so rapid that they cannot be distinguished but the corpuscles in the neighbourhood are seen moving. In a few minutes one or more breaks off and if fortunately a female gamete is in the same field the loose flagellum (mikrogamete) can be seen entering the female. The pigment of the latter shews active movements at this stage.

Vermiculi—The formation can readily be observed on the slide A conical projection forms at one point of the fertilized gamete (copula) This elongates slowly and gets curved forming an egg shaped or spindle shaped mass The conical portion eventually separates leaving behind the remains of the cell with the pigment The vermiculus is thus at first unpigmented but later again it is pigmented (KOCH) In the fresh specimen the protoplasm appears vacuolated and has a nucleus which is readily stained by Romanowsky stain

Note that the vermiculus (or ookinet) shews forward rotatory and peristaltic motions The further development of the vermiculi is completely unknown

Post mortem Pigment is found in the kidney intestine bone marrow liver and especially the spleen The brain on the contrary is almost entirely free from it

It is probable that the halteridia of all birds are not of the same species Inoculation from one bird to another is extremely difficult if not impossible This may be due to the fact that the parasites in the blood are in all sexual forms In monkeys we appear to have a parallel condition viz gametes only in the blood the asexual forms being unknown

3 *H Kochi* These haemamoebae occur in monkeys The forms usually met with are sexual forms Asexual forms resembling young *malaria parasites are very rare Flagellation* can be seen in fresh specimens The parasites in the fresh film are spherical pale bodies

containing brownish yellow pigment On staining two types can be distinguished The male (mikrogametocyte), pale homogeneous blue with much chromatin the female, deep blue, granular, with little chromatin

No temperature changes occur in the infected animals The infection is not transmissible by inoculation (*cp* halteridium)

Post mortem —The spleen is pigmented the capsule thickened Pigment also occurs in the marrow

PARASITES IN BATS

DIONISI has described in bats parasites which have a general resemblance to malarial parasites but almost certainly have no real relation thereto He distinguishes the following forms —

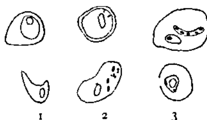


Fig 68 (1) *H Murinus (above) medium size (below) free form* (2) *H Melanipherus (above) medium size (below) large Ovoid form* (3) *H Vesperuginis (above) irregular form (below) Ring form* (*After* DIONISI)

4 *H Melanipherus* (polychromophilus melanipherus) —This occurs in the blood of *Miniopterus Schreibersii*, and is so called on account of

---

Koss u Z sch f Hyg Bd XXXII

its staining reactions with ROMANOWSKY, and because it is pigmented. It somewhat resembles the quartan parasite (Fig. 68).

5. *H. Murinus* (polychromophilus murinus).—Found in the blood of *Vespertilio murinus*. It is also pigmented. It shews like the former a variety of polychrome effects with ROMANOWSKY. In this as in the former DIONISI figures a great variety of forms (Fig. 68).

6. *H. vesperuginis* (achromaticus vesperuginis).—In the blood of *Vesperugo noctula*. The young forms resemble those of the malignant tertian parasite (Fig. 68). It occurs in large numbers in the blood but forms no pigment during its development. It produces considerable anaemia and degenerative changes in the red cell.

How bats are infected is quite unknown.

The parasite found differs according to whether the animal is hibernating or not.

7. [*H. bovis*].—Parasites in the blood of cattle described by KOLLE in South Africa. They have a general resemblance to malaria parasites but are quite distinct from *Piroplasma bovis*. They produce remittent fever and severe anaemia, but not haemoglobinuria. KOLLE also describes pigment in red cells (independently of parasites) but what this means is not clear.

8. *H. Metchnikovi*.—Found in the blood of *Trionyx Indica* or *Chitra Indica*, a large fresh water tortoise in many Indian rivers. All adult specimens of this tortoise from the Jumna were infected.

K Z f f Hyg 898

W

The parasite resembles *H Danilewskyi* (*halteridium*) in that two forms are easily distinguished in the blood—(1) a hyaline form with large pigment grains staining very slightly with methylene blue (2) a granular form with fine pigment staining deeply with methylene blue These forms correspond to the male and female gametes respectively In one of SIMOND's figures *it is interesting to observe a male and female gamete in the same red cell*, which so far as we know, has never been observed in the case of *H Danilewskyi* But besides these pigmented forms there are also found unpigmented forms,



Fig 69 H Metchnikovi Gametes and Vermicule

which have the typical gregarine look that is to say curved, worm like bodies The exact relationship of the haemogregarine to the haemamoebae forms is not understood SIMOND however points out that halteridium has a vermicule stage and there is the possibility of the relationship being similar in this case (Fig 69)

GENUS HAEMOGREGARINA

The haemogregarines are unpigmented unicellular organisms, which, at one stage of their development have a worm like form They occur

as endoglobular parasites and also as free forms in the plasma The vermicule stage may be both endoglobular and free The sexual and asexual cycles occur as far as is known in the same host They occur in fish amphibians and reptiles but not in mammals and unlike the gregarines not in invertebrates They are so far as is known non pathogenic and they cannot be transmitted by inoculation from one animal to another The cycle of development as far as it is known will be described under the various species

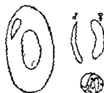


Fig 70 H Ranarum (= D epranid um Ranarum) Young form Gametes free in the Plasma and Tissue forms in Spleen (Partly after Minchin)

1 *H Ranarum (= Lankesterella Ranarum)* Found in the blood of *Rana Esculenta* (edible frog) This species includes according to LAVERAN two species *H princeps* and *H monilis* described by LABBE Here as in other species of Haemogregarines the sexual and asexual cycles occur in the same animal The cycle of development is as follows —

(1) *Sexual Form or Schizonts* —These are endoglobular four to eight μ in length Increase in size takes place and eventually they become spherical and divide into a number of segments

(schizonts) According to some observers segmenting forms are only found in the spleen

(ii) *Sexual Forms*—Free in the plasma twelve to fifteen μ long These are male and female and are characterized by the same general differences as other gametes the male mikrogametocyte is slender and finely granular the female makrogametocyte is fat and coarsely granular

(iii) A mikrogamete in the form of a small mass of chromatin separates off and fertilizes the (now) makrogamete

(iv) A zygote results which is at first motile This becomes encysted as the

(v) Oocyst which is found in the *epithelial cells* of the intestine This passes out eventually in the faeces of the frog Sporoblasts are formed as in the malarial cycle and from these result

(vi) *Sporozoits*—These would gain access to a fresh frog which had swallowed an oocyst Hintze has shewn that frogs confined in pools are especially liable to infection



Fig 71 *H Splendens—Adult Form with Refractile Granules*

H Splendens (= Dactylosoma Splendens)—Found in the blood of *R esculenta*

The following forms are figured by LABBE (Fig 71) —

(i) Amoeboid forms

(ii) Forms resembling in shape a finger glove

(iii) Segmenting forms as in *Haemamoeba Relicta* (Proteosoma)

The protoplasm contains no pigment but refractile granules

This differs from the typical development of haemogregarines and it is probable that its position requires revision According to HINTZE it is a variety of *H Ranarum*

3 *H Magna*—Described by GRASSI and FELETTI in *R esculenta* MINCHIN thinks it may be the makrogamete of *H Ranarum* or *H monilis*

4 *H Riedyi*—Occurs in a Californian Salamander *Batrachoseps attenuatus*

5 *H Stepanowi*—It is found in the tortoise *Cistudo Europaea* This may be taken as the type haemogregarine It presents the following forms (Fig 72) —

(i) Reniform parasites ten to fourteen μ long Curved and thickened at each end granular non pigmented Intermediate forms occur between this and the next developmental stage

(ii) Vermicule forms also endoglobular but after examining a fresh specimen of blood for some time free forms are seen thirty to forty μ long and three to four μ broad These are actively motile and constrictions can be seen travelling down their length during the motion Young forms and reproductive forms are not seen in the circulation These are

found in the liver The reproductive forms are at first endoglobular but later free They occur as

(iii) Ovoid forms, ten to sixteen μ long by four to six μ broad, shewing as many as six nuclei (chromatin masses) The protoplasm finally segments and there is formed

(iv) An actively amoeboid young form

The spores that are found in the kidneys of tortoises belong according to I a rr iv not to the haemogregarine at all but are those of a *Myxosporidium (M Danilewskyi)*

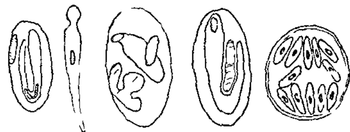


Fig 7 H Stepanowi Endoglobular and free Vermicules H Lacertarum shewing disintegration of Nucleus of Red Cell H Lacertae H Lacertarum Cyst with Makromerozoits

6 *H Lacertarum (=Karyolysus Lacertarum)* (Fig 72)—Found in the blood of *Lacerta agilis L muralis* and *L ocellata*

The parasite has a more compact form than some of the other haemogregarines They exert a marked action on the red cells which become much enlarged and anaemic, and as the name of the species implies a disintegrating

action on the nucleus is one of its effects The nucleus is either pressed to the side or broken up into fragments

The parasite in its endoglobular stage becomes encysted and is then called a *cytocyst* Further it appears as if at this stage sexual differences appeared for some of these cysts divide up into about a dozen *macromerozoits* (Fig 72) while others divide up into twice as many or more *micromerozoits* Corresponding to these we have free forms in the liver twelve by three μ and eight by two μ respectively



Fig 73 (1) H Mesnili showing characteristic shape of Vermicule (2) H Lacazei showing characteristic hook of Vermicule and two bright Granules (after SIMOND) *(3) H Ziemanni in Blood of Blennie (after* MINCHIN)

7 *H Lacazei* (= *Haemocytozoon clavatum*) In the blood of lizards The vermicules have a peculiar shape (Fig 72) Here also cyst formation has been described in the spleen by LABBE

8 *H Mesnili* —In the blood of a tortoise *Emys tectum* (Fig 73)

Amoeboid forms, reniform and vermicule forms occur Besides these free merozoits but their origin is obscure The form of the vermicule is characteristic at one stage of its development

9 *H Laverani*—In the blood of Indian tortoises *Cryptopus granosus* Similar forms occur to those of the last species The vermicule is characterized by a blunt hook like appendage and the presence of two bright granules

The parasite is endoglobular in all its stages

10 *H Bigemina*—Discovered by Laveran in the blood of blennies A vermicule form occurs free in the plasma

The endoglobular parasite divides by simple binary fission In fishes we also have *H delagei* in two species of ray and *H simondi* in the sole

These must suffice to give an idea of the characteristics of these parasites but they by no means exhaust the total for haemogregarines have been described in thirty one species of snakes seven lizards three crocodiles eleven tortoises and turtles, so that ample material for study exists in the tropics The subject is at present however in considerable confusion, and much further work is required

Genus Piroplasma

SPECIES	HOST
P Bovis	Cattle (Texas fever organism)
P Canis	Dogs (Italy Senegal France)
P Ovis	Sheep (Italy Roumania)
P Equi	Horse (S Africa)
P Hominis	Man (producing spotted fever)

1 *P bovis*—The parasite of Texas fever of cattle clearly does not belong to the proper group of malaria parasites although it occurs in the red cell It forms no pigment and differs in its development from the malaria parasite (Koch)

The parasites are two to four μ in length one to two μ in width Two forms occur in the circulation—(i) a spherical or ovoid form (ii) a piriform parasite in pairs These are characteristic and give the name Intermediate stages between (i) and (ii) occur The spherical forms show a chromatic particle and closely resemble young rings The chromatic body (nucleus) divides into two portions one going to each end the parasite elongates and by this means the piriform body is got The piriform parasites are two to three μ long and about one μ in diameter

The number of parasites in the peripheral circulation is proportionate to the severity of the disease—one to two per cent of corpuscles are infected at the end of the disease five to ten per cent (or even twenty five to thirty per cent) The number in the blood is not so great as the number in the spleen (ten per cent) liver (thirty per cent) and especially kidneys (eighty per cent)

Free parasites are found in the blood in the later stages of the disease but especially in the kidneys

Post mortem —Haemorrhagic oedema about the stomach kidneys and retroperitoneal tissue Intense hyperaemia of the spleen and kidneys the latter are nearly black Haemorrhagic erosions Ulcers in various portions of the alimentary canal Ecchymoses in pelvis of kidney

Transmission by Ticks —According to MOTAS (Bucharest) *Piroplasma ovis* can be transmitted by transferring *adult* ticks from an infected to a non infected animal He did not succeed in transmitting the disease by larvae or nymphae developed from ticks taken from infected animals

This is in direct opposition to the classical researches of SMITH and KILBORNE on *Piroplasma bovis*, corroborated by KOCH. SMITH and KILBORNE hatched young ticks from the eggs of ticks that dropped off infected cattle. It is these young ticks that communicate the disease.

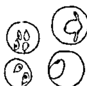


Fig. 74. *Piroplasma Canis (left) typical Piriform Parasites. (right) Amoeboid forms.*

2. *Piroplasma canis.*—The parasite is morphologically identical with *Piroplasma bovis*. It is a strictly specific parasite and has not been transferred to any other animal than the dog. Native dogs in the tropics may harbour the parasite without shewing any symptoms. The disease exists in France, Italy, South Africa, etc. In the chronic forms the parasite is rare in the circulation but in the acute form with high fever, icterus, and haemoglobinuria, the parasite (typical pirosoma form) is found with great ease in the blood. Four to six parasites often occur in each cell. Kidney blood post-mortem is extremely rich in parasites. Young dogs two to twelve weeks old are the most easily infected by intravenous injection. The tick *Dermacentor reticulatus* is supposed to convey infection in Europe and *Haemaphysalis laechi* in South Africa. Both these ticks appear to pass their larval stages on other hosts than the dog.

3 *Piroplasma ovis.*—The disease in Hungary is known as carceag. BABES considers that the organism forms a connecting link between the bacteria and protozoa. Sheep that have recovered have a marked immunity.[c]

4 *P. (kochi).*—African Coast Fever described by Koch is an exceedingly virulent form of piroplasma infection in Rhodesia and South Africa; eighty to ninety per cent. of infected cattle die. A peculiarity of the disease is that the anaemia is slight and correspondingly haemoglobinuria rare. The parasite is smaller than that of the piroplasma of Texas fever. The parasites are disc-shaped or leaf-shaped and as the disease progresses may be found in almost every cell. Pear-shaped organisms are rare. It is suspected that *Rhipicephalus decoloratus* or the blue tick transmits the disease, for this tick transmits also Texas fever in South Africa.



Fig. 75. Atypical forms of *Piroplasma* (After LAVERAN.)

It is in this form of bovine piroplasma especially that atypical forms have been described by THEILER, and subsequently more fully by LAVERAN.

(1) Forms resembling straight or curved bacilli one to three μ long. They are thicker at one end which contains a chromatin particle. One to four may occur in the same cell.

(ii) Forms resembling cocci, singly or in pairs. Two to four may occur in the same cell.

It is important to note *that together with the atypical forms typical forms are always found* though these latter may be rare.

These atypical forms occur in the severe cases. Similar cocci like forms described by SMITH and KILBORNE in Texas fever occurred in the *slight* cases. The post mortem lesions characteristic of this form of pirosomal disease are local infarcts in various organs.

5. *Piroplasma hominis*.—This species of piroplasma is responsible for the disease known as "spotted fever" (fleck typhus) occurring in Montana and Idaho U.S.A. and also in Egypt. As the name implies there accompanies the fever an eruption of spots. The mortality in the United States is as high as seventy to eighty per cent. It is much less in the cases described in Alexandria, Egypt. It is possible that they are not the same diseases and the subject requires elucidation. In the Montana disease piriform ring shaped and cocci like forms occur.

6. *Piroplasma Equi* (LAVERAN).—Found in horses in South Africa.

TICKS

Life History.—The female after satiating herself with blood falls to the ground and in a few days or weeks lays eggs.

Eggs.—The eggs are laid in masses of several thousands (*Ixodidae*) of some hundreds (*Argasidae*). The process lasts about a week. They are small oval opaque bodies. They may take weeks or months to hatch out. *From the eggs is developed*—

The Larva.—These are hexapod. They cling to blades of grass, etc., and may do so for several months before attacking a host. The larval stage lasts six to ten days. The moult then takes place and there emerges from the skin—

The Nymph.—These are octopod. They resemble adult females. They have respiratory stigmata but no sexual organs. The nymphal stage last seven to ten days. The nymph moults and there emerges—

The Adult. These again attach themselves to the host and in a few days copulation takes place. The female gradually distends and remains attached for about nine to eleven days. The female then drops off. What the male does is uncertain. The female's whole cycle *on the host* is thus twenty two to thirty one days. The *entire* life cycle takes probably about two to three months.



Fig. 76. Eggs, Larva and Adult Tick. (After Massé.)

The dimensions of the various stages of *I. Ricinus* are:—

Eggs	0.4 by 0.3 millimetres
Larva	0.6 by 0.4 millimetres
Nymph	1.3 by 0.6 millimetres
Adult	2.5 by 1.5 (male) millimetres
Adult	10-11 by 6-7 (female full grown) millimetres

Ticks have a predilection for certain hosts, but the same tick may be found on different animals, and further, the larval and adult stages may be passed on different animals

Anatomy

1 *The Rostrum*, capitulum or head is the small anterior projecting portion, it is joined on by a short neck to the scutum on its ventral surface is seen—

2 *The Labium* or hypostome, a bi-laterally symmetrical structure furnished with a number of teeth directed backwards The number of rows of teeth and their disposition are very important in classification

3 *The Mandibles* lie dorsally to the labium The terminal portion (digit) terminates in two or three processes, apophyses which bear hooked teeth directed backwards They are important in classification

4 *The Mandibular Sheath* lies above the mandibles The anterior extremity is notched corresponding to the two halves of the sheath (2) (3) and (4) *form the piercing organ or haustellum*

5 *The Palpi* are four jointed and form a kind of sheath for the haustellum The shape of the palpi, their spines and processes are of the greatest importance in classification

6 *The Scutum* is a dorsal structure, situated behind the base of of the rostrum It is a hard leathery plate In the male it practically covers *the whole* of the dorsum In the female it is confined to a roughly triangular anterior portion of the dorsum The males are thus

readily distinguished from the females It is absent in the *Argasidae*

7 *The Porose areas* are dorsal structures forming two oval depressions one on each side of the middle line at the base of the rostrum They are most conspicuous in the female but exist in both sexes

8 *The Eyes* not always present are small almost globular structures situated laterally at the margin of the scutum in the *Ixodidae* or as punctiform structures on the supracoxal fold of the first leg in the *Argasidae*

9 *The Stigmata*—Situated ventrally and laterally behind the level of the fourth legs in the *Ixodidae* open into a stigmal plate or peritreme In the *Argasidae* they lie between the third and fourth legs The shape of the plates are important in classification

10 *The Anus* is a little way in front of the posterior ventral margin It has a valve

11 *The Anal plates* or *clypei* four in number on either side of the anus in the male They are used for classifying Not always present

12 *The genital orifice* is in the middle line a little way behind the rostrum

13 *The Legs* are six in the larvae eight in the nymphs and adult The claws have ventrally a well marked pad or *pulvillum* The coxae (with which the trochanters articulates) may have spines or teeth or larger shields These are used in classification

Classification of Ticks

Ticks are divided into two families—

1 *Argasidae* scutum absent
2 *Ixodidae* scutum present

The *Ixodidae* are divided into two sub families—

1 *Rhipicephalinae* —Palpi not longer than broad rostrum short Anterior portion of body emarginate to receive the rostrum
2 *Ixodidae* —Palpi longer than broad ros trum long Anterior portion of body straight or emarginate

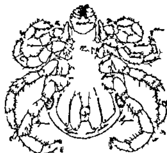


Fig 77 Tick under surface showing anatomy and parts used for classification (*After* SALMON *and* STILES)

The *Rhipicephalinae* are the most important from our standpoint as to the genus *Rhipicephalus* belong most of the ticks that are known to trans mit parasites The various genera are *Rhipice phalus [Boophilus] Haemaphysalis* and *Derma centor*

GENUS RHIPICEPHALUS

Eyes present Base of rostrum hexagonal (dorsally) forming on each side a projecting angle Palpi short and broad Stigmata, comma shaped clypei two pairs in the male Coxae i, two large teeth The genus includes nearly thirty species Some of these including the carriers of piroplasma, we may classify in the following way —

Classification of part of Genus Rhipicephalus

Species	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	Remarks
R. Annulatus	Extend to post. lat. margin	Eight rows of teeth	Int. apophysis bicuspid	Extends to post. margin	Absent	= *B. Annulatus* = *B. Bovis*. Transmits American Texas Fever
R. Caudatus	ditto	Ten rows	—	ditto	Distinct	
R. Evertsi	ditto	Six rows	Tricuspid	Not extending to post. margin	Small	= Red Tick South Africa
*R. decoloratus**	ditto	Six rows	Bicuspid and rounded [illegible]	Extending to post. margin	Distinct	Transmits Texas Fever in South Africa = Blue Tick of S. Africa and Rhodesia
R. Australis	ditto	Eight rows	Tricuspid and rounded [illegible]	ditto	—	Transmits Texas Fever in Australia
R. Sanguineus	ditto scutum with unequal punctations	Six rows	Tricuspid	Not extending	—	Europe, Africa, America, commonest tick in Rhodesia
R. Pulchellus	Become obsolete in middle scutum white ♀ Black and white ♂	—	—	—	—	Zanzibar

* [illegible]

Genus Boophilus

Not admitted by Neumann as a genus

Genus Haemaphysalis

Eyes wanting. Base of rostrum rectangular, twice as long as broad, palpi conical. Second segment of palpi has a well marked lateral conical projection. Stigmata comma shaped or circular. Anal shields absent in male. Coxa i not bifid. Coxa iv in male a well marked spur. There are about twenty six species.

H. Leachi (South Africa).—The dog is the usual host. Possibly transmits *Piroplasma canis*, occurs also on cattle.

Labium.—Four rows of teeth in ♀, five rows in ♂.

Palpi.—Dorsal surface as broad as long. Second palpal segment has a sharp lateral spine.

Coxa iv.—Has a tuberosity.

Genus Dermacentor

Eyes present. Base of rostrum broader than long. Palpi short and thick. Stigmata comma shaped. Anal shields absent in male. Coxa i bidentate in ♂ and ♀. Scutum ornamented. About twenty four species.

D. electus is the American dog tick.

The subfamily *Ixodinae* consists of five genera, *Ixodes*, *Eschatocephalus*, *Aponomma*, *Amblyomma* and *Hyalomma*.

Genus Ixodes

Eyes absent. Palpi long. Tarsi without terminal spurs. Anal groove surrounds anus anteriorly and opens posteriorly. Scutum in male does not cover the body laterally and posteriorly. Stigmata oval in ♂, circular in ♀. Male ventrally covered with six shields: two lateral embracing the origins of the legs and the stigmata; one median between the genital opening and the anus; two on each side of the anus (perianal); and one triangular posterior shield carrying the anal orifice at its anterior corner. Female has dorsally three longitudinal grooves on the abdomen; ventrally two bell-shaped grooves, the first has its apex at the vulva, the second at the anus. There are a large number of species.

1. *I. ricinus*.—The castor bean tick is common on sheep, goats, cattle. Europe.
2. *I. hexagonus*.—The European dog tick.

Genus Eschatocephalus

Eyes wanting. Rostrum long. Palpi piriform ♂, claviform ♀. Anal grooves as in *Ixodes*. Stigmata circular in both sexes. Legs very long. Dorsal and ventral chitinous thickenings in the male, fine grooves in the female. There are seven species.

Genus Aponomma

Eyes wanting. Anal groove surrounds anus posteriorly and opens anteriorly. Anal plates absent. Base of rostrum pentagonal. Scutum

covers the dorsum entirely usually marked with green spots Stigmata, comma shaped Female scutum shorter than broad three green spots The species are parasitic on reptiles

Genus Amblyomma

Eyes present conspicuous Anal groove as in *Aponomma*, anal plates absent Rostrum long Scutum often has coloured designs Stigmata usually triangular nearly always eleven posterior marginal festoons in the male There are over eighty species

A variegatum—Is frequent on cattle in Rhodesia

Genus Hyalomma

Eyes present conspicuous Rostrum long Anal groove opens anteriorly Body elongate oval Colour deep-brown Male two pairs of ventral shields two perianal large, triangular, and two small external Scutum covering nearly the whole of the dorsum Crenellated or festooned posteriorly Male stigmata comma shaped with a long tail female stigmata with a short tail Three species only

H Aegyptium—Attacks cattle especially, also dogs and cats Occurs in Egypt North and South Africa

Argasidae—Scutum absent rostrum inferior (except in larva) Stigmata between third and fourth legs Pulvillum of tarsi wanting in adult Palpi free short filiform four segments Legument leathery without dorsal or ventral shields Sexual dimorphism not marked

Genus Argas

Eyes absent. Rostrum which is concealed by the cephalothorax is situated at least its own length behind the anterior margin. No projecting hood. Body oval or orbicular. The species are nocturnal in their habits; infest birds mainly. There are eleven species. A species of Argas is the transmitter of spirillar fever of poultry.

A. reflexus infests pigeons. European.

A. persicus—Garib Guez of Persia. The bite is said to produce severe local and constitutional effects.

A. tholozani = Kéne of Persians; similar effects ascribed to it.

Genus Ornithodorus

Eyes present or absent. Rostrum hidden under a projecting beak (hood) close to the anterior margin of the body, so that the tips of the palpi are visible from above. Lateral borders of body generally straight, sometimes concave; tegument mammillated.

O. moubata—Carrapato tick of Tete on the Zambesi. The bite is said to occasion severe local and general disturbances. It is the cause of tick fever in Uganda (Christy).

YELLOW FEVER

Myxococcidium Stegomyiae

The following is a brief summary of the work done by the Americans (Parker, Beyer and Pothier) on the parasite of yellow fever.

LITERATURE

1 *Cattle Ticks of the United States* Salmon and Stiles Washington 1902 An excellent compendium on ticks many illustrations Price a few shillings

2 L G Neumann Révision de la famille des Ixodidés *Memoires Soc Zool France* Complete monographs

3 *Texas Fever* Theobald Smith and F L Kilborne 1893 Washington Bulletin No 1

4 Wasielewski *Sporozoenkunde*

5 *The Sporozoa* Minchin in Lankester s Zoology

6 *A Monograph of the Tsetse Flies* Austen (Nat Hist Brit Mu)

Chapter XXVII

THE TRYPANOSOMIDAE

TRYPANOSOMATA AND TRYPANOPLASMATA

The *Trypanosomidae* comprise two genera—(1) *Trypanosoma* (2) *Trypanoplasma* The genus *Trypanosoma* is characterized by the possession of a longitudinal undulating membrane the thickened border of which takes its origin posteriorly from a centrosome, and terminates anteriorly in a free flagellum Division takes place longitudinally The genus *Trypanoplasma* has two flagella one anterior the other posterior Both arise from one centrosome the anterior forms the thickened border of the undulating membrane the posterior flagellum curves around the posterior end of the parasite and then is prolonged into a flagellum about equal in length to the anterior one

The *Trypanosomidae* occur in fish amphibia reptiles birds and mammals Most of these are very incompletely known and it is only some species in mammals that have been at all closely studied We may enumerate the following species —

Trypanosoma rotatorium (synonym = *Trypanosoma Sanguinis* (GRUBY) —In the blood of frogs (*Rana esculenta* *Rana temporaria* *Hyla arborea* etc) forty to eighty μ long five to ten μ

LITERATURE

C[illegible] of the United States. [illegible]

2. L. G. [illegible]

3. [illegible] and F. L. [illegible]

4. W[illegible]

5. [illegible] Z[illegible]

6. [illegible]

Chapter XXIII

THE TRYPANOSOMIDAE

TRYPANOSOMATA AND TRYPANOPLASMATA

The *Trypanosomidae* comprise two genera—(1) *Trypanosoma*, (2) *Trypanoplasma*. The genus *Trypanosoma* is characterized by the possession of a longitudinal undulating membrane, the thickened border of which takes its origin posteriorly from a centrosome and terminates anteriorly in a free flagellum. Division takes place longitudinally. The genus *Trypanoplasma* has two flagella, one anterior, the other posterior. Both arise from one centrosome; the anterior forms the thickened border of the undulating membrane; the posterior flagellum curves around the posterior end of the parasite and then is prolonged into a flagellum about equal in length to the anterior one.

The *Trypanosomidae* occur in fish, amphibia, reptiles, birds, and mammals. Most of these are very incompletely known, and it is only some species in mammals that have been at all closely studied. We may enumerate the following species:—

Trypanosoma rotatorium (synonym = *Trypanosoma Sanguinis* (GRUBY)).—In the blood of frogs (*Rana esculenta*, *Rana temporaria*, *Hyla arborea*, etc.), forty to eighty μ long, five to ten μ

broad Flagellum ten to twelve μ long Surface of the body striated longitudinally (Fig 79 and Pl II, fig 3)

Ts Carassii (MITRAPHANOW)—Besides the forms with undulating membrane and flagellum disk-like forms are described In the blood of fish (*Carassius vulgaris*), in the tench (*Tinca vulgaris*) Also described in the blood of the stickleback pike etc (Fig 79)

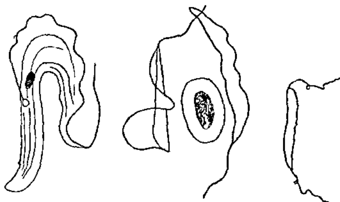


Fig 79 T Rotatorium T Cobitis T Carassii Tp Danilewski Left to right (After LAVERAN *and* MESNIL MITRAPHANOW *and* DANILEWSKI)

Ts Eberthi (KENT)—In the guts of fowls, ducks geese especially in the caecum and ileum This is not a blood parasite so far as is known

Ts Cobitis (MITRAPHANOW)—In the blood of the mud-fish (*Cobitis fossilis*) Thirty to forty μ long, one to two μ broad Flagellum ten to fifteen μ It is long and thin Forms without an undulating membrane are described and also without a flagellum (Fig 79)

Ts soleae (LAVERAN) —In the blood of the sole

It is however, the trypanosomes of mammals that are of the greatest importance producing as they do well known and fatal diseases Not all trypanosomes of mammals are however, pathogenic Trypanosomes are bodies easily detected in fresh blood with a one sixth or one seventh lens They are actively motile and may be seen displacing the red cells by their motions As they come to rest the undulating membrane and flagellum are visible They are bodies about twenty μ long In stained specimens (ROMANOWSKY) an oval nucleus lies about the middle of its length and near the blunt posterior end a small stained particle is clearly



Fig 80 T Lewisi—(1) Dividing form with two centrosome (2) Adult form (3) Young forms resulting from division (fresh preparation)

seen the centrosome From this in certain species at any rate the flagellum starts and can be seen as a distinct wavy thick red line extending the whole length of the organism and continued beyond is the long (anterior) free flagellum The portion (unstained) between this external wavy margin and the blue stained body of the organism is the undulating membrane (Fig 80)

(*c*) Branched hairs on the upper surface only of the *arista* (the arista is an appendage of the terminal segment of the antenna) (*vide* Fig 82)

(*d*) Male genitalia (hypopygium) characteristic

(*e*) Membrane of wing grooved, not smooth, as in other genera

(*f*) Wings closed flat over one another like a scizzors

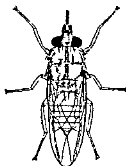


Fig 83 *Glossina shewing scissors position of Wings when at rest* × (*After* AUSTEN)

2 *Stomoxys*

(*a*) Greyish flies with black markings (smaller than *glossina*) found on men and cattle

(*b*) Palpi exceedingly slender and short, not protecting the proboscis

(*c*) Fleshy labella, small but visible

(d) *Arista* distally forms a fine hair
(e) Wings diverge widely S *Calcitrans* is a common European species

3 *Haematobia*
(a) Small mottled flies e g horn fly of United States
(b) Palpi distinctly shorter than proboscis expanded at the tips
(c) Tip of proboscis bears a few hairs
(d) The modified flesh labella easily visible
(e) The arista has hairs on under and upper surface



Fig 54 Stomoxys showing resting position of Wings × (After Austen)

4 *Lyperosia*
(a) They occur in great numbers on for instance camels
(b) By some regarded as a sub genus of *Haematobia*
(c) Palpi form a complete sheath for the proboscis and flattened as in *Glossina*
(d) Palpi not expanded at the tip
(e) Closely allied to *Glossina* but differing in the wings

5 *Bdellolarynx*
(a) Proboscis resembles that of *Haematobia*

(b) Palpi not expanded at the tip
(c) Arista feathered only on upper surface
(d) Has a prominent *Epistoma* (thus differing from other genera) [The tip of the Epistoma is the point from which the total length to the tip of the abdomen is measured]
(e) First posterior cell of the wing closed before the margin (in *Glossina* it is open)

Other blood sucking flies that may be confused with the tsetse flies belong to a different family, viz *Tabanidae* (horse flies, clegs, or gad flies) In this family those resembling tsetse are the genera —



Fig 85 Haematapota shewing resting position of Wings × (*After* AUSTEN)

1 *Hematapota*
(a) They are about the same size as large tsetse flies—eleven mm long
(b) The abdomen is not banded
(c) The wings diverge at the tip but meet at the base (*vide* Fig 85)
(d) *The antennae project horizontally for wards and are conspicuous*
(e) The grey small *Haematapota* of English lanes in summer is a familiar species

2 *Pangonia*

(a) Characterized by the extreme length of the proboscis in some species three to four times the length of the body

(b) The Seroot fly of Nubia is a species of *Pangonia*

(c) Antennae projecting (family character)

Genus Glossina

Abdomen generally but not always has pale but well marked dark-brown bands interrupted in the middle

1 Dull coloured brownish flies seven to twelve mm long (excluding proboscis and wings)

2 Wings in resting position closed flat one over the other scissors like projecting beyond the abdomen

3 Proboscis enclosed in palpi projecting horizontally in front

4 Base of proboscis suddenly expanded into a large onion shaped bulb

5 Arista feathered on upper side only

6 Male genitalia (hypopygium) highly characteristic oval and tumid with a vulviform median groove (anus) running from anterior margin to beyond the middle Sex easily distinguished by this mark

7 Wings absolutely characteristic especially in the course of the *fourth longitudinal vein* (vide Fig 82, iv) The anterior transverse vein is

very oblique The bend in the course of the fourth vein before it meets the anterior transverse vein is absolutely diagnostic

Larva and Pupa —According to BRUCE, tsetse flies (or at least one species) do not lay eggs but extrude a yellow-coloured larva After a few hours this changes into a pupa The pupa is six mm long and three mm broad It consists of twelve segments The twelfth segment is produced into two large lips, enclosing a pit, the site of the respiratory stigmata in the larva At the anterior end is a longitudinal groove through which the fly eventually emerges

CLASSIFICATION OF SPECIES

(1) Hind tarsi entirely dark

Gl palpalis —Darkest of all species of *Glossina* Third joint of antenna dusky brown to cinereous black

Gl pallicera —Third joint of antenna orange buff Front in both sexes *narrower* than in *Gl palpalis* In ♂ the arista is stouter and longer than that in *Gl palpalis*

(2) Hind tarsi not entirely dark

Small species length not exceeding ten and a-half mm Last two joints of front and middle tarsi have sharply defined dark-brown tips

Gl morsitans —

1 Smaller than *G longipalpis*
2 Head narrower
3 Front paler and wider
4 Eyes in ♂ and ♀ distinctly converging towards vertex

5. Abdominal bands less deep pale hind margins of segments therefore deeper
6. Hypopygium in ♂ larger paler somewhat more oval in outline and clothed with fewer hairs
7. Tip of ♂ abdomen less hairy laterally
8. Bristles on sixth segment in ♂ stouter and more conspicuous than in *longipalpis*

Gl longipalpis Small Species —First two joints of front and middle tarsi entirely pale

G pallidipes—*Large species* Length at least ten and a half mm (in this respect they contrast markedly with the other small species)

G longipennis —

1. Thorax with four sharply defined dark-brown oval spots
2. Ocellar spot dark brown very *conspicuous* compared with the body
3. Proboscis shorter than in *G fusca* and *relatively* shorter compared with the body than in any other species
4. In both sexes the *front* is broader than in *Gl fusca*

G fusca—Thorax without spots

T Evansi—The trypanosome of Surra A common disease in many parts of India e g Bombay at certain seasons especially though probably always in a latent condition It is possible that its increase at a particular time is associated with the prevalence of a biting fly Surra is characterized by a similar train of symptoms to those of Nagana Rogers states that the disease in India is conveyed by *Tabanidae* (horse

flies). If this is so, the probability of Ngana being also conveyed by other biting flies than the genus *Glossina* seems likely.

Whether the 'surra' of camels in India is produced by the same trypanosome there is no evidence to shew.

LAVERAN and MESNIL, who have recently been able to make a comparison of *T. Brucei* and *T. Evansi*, state that *T. Brucei* is shorter and more compact than *T. Evansi*. The movements of *T. Brucei* are also less extensive. The posterior end of *T. Brucei* is also blunter than that of *T. Evansi*. The free portion of the flagellum is shorter in *T. Brucei* than *T. Evansi* and the protoplasm of *T. Brucei* has more numerous and larger granules than that of *T. Evansi*. The nuclei and the centrosomes are morphologically indistinguishable. Further, the mean length of *T. Brucei* is less than that of *T. Evansi* and the width of *T. Brucei* is greater. The distinction between Surra and Ngana is, however, best proved by the fact that an animal immunized against Ngana is yet susceptible to inoculation with Surra.

Trypanosoma Equinum (Mal de Caderas).—In Central and South America. A disease affecting horses.

The symptoms—remittent fever, oedema, wasting—resemble those of Ngana and Surra. Most characteristic is the paralysis of the hind legs from which the disease takes its name.

It runs a chronic course, two to six months. In donkeys six to twelve months. There is occasionally haemoglobinuria.

Mice rats, rabbits dogs etc (guinea pigs rarely) are susceptible Incubation period five to eight days Horned cattle are refractory

It is thought that the infection is transmitted by a biting fly *(Stomoxys calcitrans)* The parasite morphologically resembles *T Brucei* In the latter the centrosome is however larger In *T Equinum* it is so small that its existence has been denied Moreover an animal immunized against *T Equinum* is still susceptible to *T Brucei*

T Equiperdum (Fig 81) —This trypanosome is the cause of the disease among horses in Algeria known as *Dourine* In asses the symptoms are slight In horses and especially stallions the symptoms are much more marked It is conveyed as far as is known under natural conditions by coitus only and not by means of flies

In eleven to twenty days after coitus oedematous swellings of the genitalia appear

In forty to fifty days characteristic plaques on the skin These are very occasionally absent as in asses but when present are pathognomonic These plaques last only one to eight days Around these there is oedema The animals become anaemic complete paraplegia sets in and death in two to ten months

Trypanosomes are most easily found in the plaques with difficulty in the blood

Post Mortem —There is inflammation of the urogenital mucosa and in two cases areas of softening have been found in the spinal cord Ruminants are refractory (to *T Brucei* they are very susceptible) Dogs which have been immunized against *T Equiperdum* yet succumb to *T Brucei* so that Dourine and Nagana appear to be distinct

T Gambiense (DUTTON) —This, the first human trypanosome to be described was discovered by DUTTON in the blood of a European in the Gambia The clinical symptoms of the case were —

(1) Irregular relapsing fever
(2) Oedema especially about the eyes
(3) Congestion of the skin
(4) Erythematous patches associated with thickening of the skin
(5) Increased pulse and respirations Loss of flesh

The trypanosomes are generally scanty in the blood They are transferable to monkeys, where they persist in small numbers for some months also to white rats Careful examinations of the blood by centrifugalization are necessary for their detection in most of these animals

How conveyed to man there is no evidence to show (*vide* Plate I)

SLEEPING SICKNESS (TRYPANOSOME)

T Ugandense (CASTELLANI) —Found in the *cerebro-spinal fluid obtained by lumbar puncture* by CASTELLANI, in seventy per cent of cases Later BRUCE found it in thirty eight cases in all, and in the blood *in twelve out of thirteen cases* Centrifugalization is necessary

There are morphological differences between *T Ugandense* and *T Gambiense according to* CASTELLANI The former has a more rounded posterior extremity The centrosome is nearer the extremity and outside the vacuole, which is larger (these differences however, may not be constant)

Symptoms —Perhaps most noteworthy in comparing it with other forms of trypanosomiasis, if indeed the disease is due to this trypanosome are —

1 A puffiness about the face
2 An enlargement especially of the cervical lymphatics (this, however Dr CHRISTY informs us is not an essential character)
3 An itchy papulo vesicular eruption of the skin

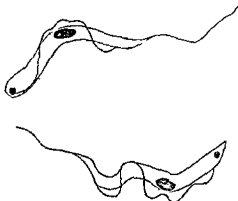


Fig 80 T *Ugandense* (CASTELLANI) *above* T *Gambiense* (DUTTON) *below* (*After* CASTELLANI)

It should be remembered that in eighty per cent of fatal cases a variety of streptococcus pyogenes was cultivated from the organs Whether or no this is to be regarded as a terminal infection and what the relation of the trypanosome to the disease may be remains to be seen

BRUCE who has continued his work finds that the distribution of the disease in Uganda

is identical with that of *Glossina palpalis* and that the trypanosome can be conveyed to *monkeys* by means of *Glossina* To CHRISTY belongs the credit of having previously shewn that the disease could not be due to *F perstans* as had been supposed



Fig 67 Hippobosca Rufipes left × *2—right natural size* (*After* THEILER)

T Theileri —This is found in the blood of cattle in the Transvaal, subject to a disease known as gal ziekte, *i e* gall sickness Length thirty to sixty five μ width two to four μ

THEILER has shewn since that a biting fly, *Hippobosca rufipes* transmits the disease

T Transvaliense —Found in the blood of oxen eighteen to fifty μ long by four to six μ broad The centrosome of this trypanosome almost touches the nucleus The undulating membrane is consequently little developed

Aino —Is the native name for a disease in Somaliland affecting camels horses asses mules It is caused by a trypanosome, and is exceedingly fatal to camels Man dog etc, in these regions are not naturally infected

Dimensions of Trypanosomata Found in Mammals

1	*T. Lewisi*	24-25 μ by 1.4 μ
2	*T. Brucei*	25-30 μ by 1.5-2.5 μ
3	*T. Equiperdum*	18-26 μ by 2-2.5 μ
4	*T. Evansi*	20-30 μ by 1.2 μ
5	*T. equinum*	20-25 μ by 2-3 μ
6	*T. Gambiense*	18-25 μ by 2-2.8 μ
7	*T. Ugandense*	18-26 μ by 2-2.5 μ
8	*T. Theileri*	30-65 μ by 2-4 μ
9	*T. Transvaaliense*	18-50 μ by 4-6 μ

Considerable variation exists between the data of observers and though these figures can be considered as approximately correct they do not suffice for distinguishing the various species

Whether it will be possible to distinguish nearly allied species morphologically, e g, *T. Brucei* and *T. Evansi* remains to be seen. Differences in the position of the centrosome and differences in staining properties hardly suffice in similar species that resemble one another closely and at present the only certain method is their pathogenic properties

Multiplication of trypanosomata takes place by longitudinal division the nucleus and the centrosome divide into two or more parts. The trypanosome becomes more or less quadrangular in form and from each centrosome a new flagellum is seen starting. Other modes of multiplication are described—conjugation transverse division formation of amoeboid forms etc. Sexual differentiation has also been suspected anyhow it is certain that in the organs of a case of

is identical with that of *Glossina palpalis* and that the trypanosome can be conveyed to monkeys by means of *Glossina* To CHRISTY belongs the credit of having previously shewn that the disease could not be due to *F perstans* as had been supposed



Fig 87 *Hippobosca Rufipes left* × —*right natural size* (*After* THEILER)

T Theileri —This is found in the blood of cattle in the Transvaal subject to a disease known as 'gal ziekte *i e* gall sickness Length thirty to sixty five μ, width two to four μ

THEILER has shewn since that a biting fly, *Hippobosca rufipes* transmits the disease

T Transvaliense —Found in the blood of oxen eighteen to fifty μ long by four to six μ broad The centrosome of this trypanosome almost touches the nucleus The undulating membrane is consequently little developed

Aino —Is the native name for a disease in Somaliland affecting camels, horses asses mules It is caused by a trypanosome and is exceedingly fatal to camels Man dog etc, in these regions are not naturally infected

DIMENSIO S OF TRYPANOSOMATA FOUND IN MAMMALS

1	*T Lewisii*	24 25 μ by 1-4 μ
2	*T Brucei* -	25-30 μ by 1 5 2 5 μ
3	*T Equiperdum*	18-26 μ by 2 - 5 μ
4	*T Evansi* -	20-30 μ by 1 2 μ
5	*T equinum*	20 25 μ by - 3 μ
6	*T Gambiense*	18 - μ by 2 2 8 μ
7	*T Ugandense*	18 26 μ by - 2 5 μ
8	*T Theileri*	30-65 μ by 2 4 μ
9	*T Transvaaliense*	18 50 μ by 4 6 μ

Considerable variation exists between the data of observers and though these figures can be considered as approximately correct they do not suffice for distinguishing the various species

Whether it will be possible to distinguish nearly allied species morphologically e g *T Brucei* and *T Evansi* remains to be seen Differences in the position of the centrosome and differences in staining properties hardly suffice in similar species that resemble one another closely and at present the only certain method is their pathogenic properties

Multiplication of trypanosomata takes place by longitudinal division the nucleus and the centrosome divide into two or more parts The trypanosome becomes more or less quadrangular in form, and from each centrosome a new flagellum is seen starting Other modes of multiplication are described—conjugation transverse division formation of amoeboid forms etc Sexual differentiation has also been suspected anyhow it is certain that in the organs of a case of

trypanosoma infection numerous strange forms can be seen, of which nothing is as yet definitely known

Inoculation—The most certain and rapid method is intraperitoneal, *e g* in the case of *T Lewisii* but subcutaneous is almost equally certain and in *T Brucei* scratch inoculations nearly always succeed

Blood Examination—If present in fair quantity there is no difficulty in detecting them fresh with a low power If very scanty, it may be necessary to centrifugalize the blood The most delicate test of a successful infection which may have resulted even though no parasites be found is a subinoculation into a highly susceptible animal

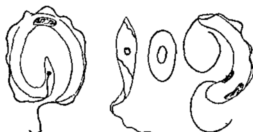


Fig 88 *T Soleae T Avium (after* DANILEWSKY) *Tp Borreli (after* LAVERAN)

Where parasites cannot be found by an ordinary examination in the blood they may, however be readily discovered in the oedematous swellings so often found in trypanosomiasis Thus it is often extremely difficult if not impossible to detect trypanosomes in the blood of a rabbit infected with *T Brucei* yet they are easily found in the oedematous fluid about the ears, muzzle, etc

Genus Trypanoplasma

1 *Trypanoplasma Borreli* —Twenty μ long three to four μ broad Each flagellum fifteen μ long It is curved in shape The undulating membrane on the convexity The anterior end is more pointed than the posterior Found in the blood of the red eye (*Leuciscus erythrophthalmus*)

2 *Trypanoplasma Danilewski* —Fifteen to twenty μ long less than one μ broad Found in the gut of leeches (possibly derived from the blood of some animal) (Fig 79)

Chapter XXVIII

FILARIA

The *Filariidae* form one of the families into which the *Nematodes* are divided. Other families in this sub order are the *Ascaridae*, *Strongylidae*, *Anguillulidae*, etc. The *Filariidae* are divided into several genera, only one of which concerns us immediately, viz., the *Filaria*, and first we shall consider those species of filaria which have their embryos in human blood. They are the following:

1. *F. Bancrofti*, syn. *F. nocturna* (MANSON)
2. *F. diurna*
3. *F. Perstans*
4. *F. Ozzardi*
5. *F. Demarquai*
6. *F. loa*
7. *F. Megalhaesi* (adults)
8. *F. Gigas* (PROUT)

The following are the characters of the embryos of each species:—

F. bancrofti.—Occurs at night in the peripheral blood; found in the internal organs by day, especially in the vessels of the lungs. *Ce. Argyrotarsis* and *Ce. Albipes* are efficient hosts.

1. Length about three hundred μ fresh (one hundred and eighty μ, stained specimens) by seven to eleven μ wide.

2 Enclosed in a sheath considerably longer than body of embryo

3 If placed rapidly beneath microscope shews at first active progressive movement (ANNETT and DUTTON) later the anterior tip of the sheath appears to become attached to the glass and movements of the embryo though active are not progressive

4 The embryo shews an anterior abruptly rounded off end and a posterior tapering for two-fifths the length There is a six-tipped prepuce and a short very fine fang

5 The stained specimen shew (i) an irregular transverse break about twenty one per cent of the length

(ii) A V shaped spot or transverse irregular break at a distance of about thirty per cent of the length from anterior end Nearly always present

(iii) An area of varying length with cells loosely arranged sixty three per cent length This is constant and represents the central aggregation of fresh specimen

(iv) An irregular sometimes oval spot often present eighty five per cent length

(v) A small central bright spot occasionally ninety one per cent length

F diurna – No differences are distinguishable between the embryos of *F diurna* and *F nocturna* either in the fresh or stained specimen (ANNETT and DUTTON) DUTTON and ANNETT have found the embryos taken from the adult female *F loa* to be practically identical with those of *F diurna* and describe a case in which infection with *F loa* was associated with embryos present in the blood during the day and not to the same extent at night

Embryos of *F. loa*, taken from the female are described by ANNETT and DUTTON.

1. Length, 208 μ.
2. Possess a sheath.
3. Spots as follows:—

(i) An oval or diamond shaped spot, twenty-four per cent. length from anterior end.

(ii) An indistinct lateral area containing scattered nuclei, thirty-seven per cent. length.

(iii) A longer portion of worm which stains badly, sometimes divided into anterior and posterior portions.

(iv) A small lateral bay, eighty six per cent. length.

F. perstans.—Embryos present in peripheral blood day and night.

1. Length, two hundred μ by four μ to five μ breadth in fresh, and about ninety μ in stained preparations.
2. Do not possess a sheath.
3. Movements extremely active and progressive movement continues for many hours. They possess the power of considerable elongation and shortening.
4. *No hooked prepuce: a fang is generally* observed protruded and retracted. The body tapers gradually for two-thirds of length and is abruptly truncated at the tail and slightly bulbous.
5. In stained specimens the following spots are made out:—

(i) A narrow irregular transverse band at distance of 26.4 per cent. length nearly always present.

(ii) A wider irregular transverse spot at thirty-six per cent length. Occasionally.

(iii) The largest of the spots an irregular transverse area sixty three per cent length. Not always present.

(iv) A very inconstant central bright speck at eighty three per cent length.

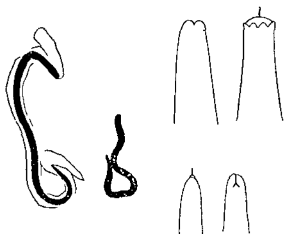


Fig. 8[illegible]. F. Bancrofti Embryo showing sheath. F. Perstans Embryo (sheathless). F. Bancrofti (Embryo) prepuce and fang (above). F. Perstans (Embryo) fang (below).

6. They are readily distinguished from the embryos of *F. Ozzardi* by their blunt tails.

7. The observations of Firket and Christy point to the fact that there is more than one species of *F. Perstans*.

F. Ozzardi.—

1. Length one hundred and seventy-three to two hundred and forty μ by four to five μ.

2 They are *sharp tailed* and sheathless

3 They have no periodicity

F Demarquaii —

1 Length two hundred and five μ by five μ

2 Tail sharp and sheathless

3 Cephalic armature, ill-developed prepuce and spine

4 A V spot exists fifty-two μ from the head (seen in wet films)

5 There is no periodicity

6 *Ce Argyrotarsis* and *Ce Albipes* are inefficient hosts

F loa —Two cases of infection with the adult *F loa* have been described in which *F diurna* occurred in the blood It is possible then that *F diurna* is the embryonic form of *F loa* (*vide F diurna* anter) On the other hand *F diurna* embryos are indistinguishable from those of *F Bancrofti* the adult forms of which are well known

F Megalhaesi —Adults only known

F Gigas —

1 Blunt tailed

2 Has no sheath

The Characters of the Genus Filaria

They are long slender worms of almost uniform breadth throughout their length The anterior extremity is rounded and the mouth often has no lips The males are distinctly smaller than the females They have an incurved or spiral tail the latter sometimes having lateral membranous outgrowths They usually have

four pre-anal and a variable number of post anal papillae and spicules which vary in size and appearance In the females the vulva opens in the neighbourhood of the mouth The host in which the filaria reaches full maturity giving rise to embryos is the definitive host the other host is the intermediary or secondary host Thus *F Bancrofti* has for its definitive host man for its intermediary host, certain species of *Culicidae*

F recondita —Definitive host dogs Intermediary host *P serraticeps* (dog flea)

ADULT FILARIAE

1 *F Bancrofti* —The adult male and females are found together sometimes in the lymphatics or in cyst like dilatations of these The embryos gain access to the circulation by the thoracic duct

2 *F diurna* — Adult form doubtful According to ANNETT and DUTTON it is *F loa*

3 *F Perstans* —The adults were found by DANIELS at the root of the mesentery behind the abdominal aorta and beneath the pericardium

4 *F Ozzardi* — Adults found by DANIELS in the sub peritoneal tissue

5 *F Demarquaii* - Adults doubtful A female form has been described differing slightly from that of *F Ozzardi*

6 *F loa* — Adults found in the subcutaneous areolar tissue also in the eyelids and beneath the conjunctiva

7 *F Megalhaesi* — Adults only known found in the left ventricle of the heart by FIGUEIRA DE SABOIA

8 *F Gigas* - Adults unknown

The following are the characters of the respective species —

F Bancrofti —Males and females found together in lymphatics

♀ 1 Length eighty-five to one hundred and fifty mm

2 Distinct neck one third width of body

3 Body plain, tapering somewhat abruptly to neck and tapering towards tail

4 Cuticle with striations

5 Tail ends bluntly, and has a small depression surrounded by two small lips

6 Mouth simple, minute, terminal

7 Ova twenty five to thirty-eight μ by fifteen μ

8 Anus ventral opening on summit of a bilobed papilla

♂ 1 Length, eighty mm

2 Body cylindrical tapering to tail No neck

3 Mouth circular simple, terminal

4 Cloaca ventral four pairs pre anal four post anal papillae (MASSON doubts the presence of these) Two unequal spicules

5 Genital tube simple Oesophagus thick walled

6 Tail vine-tendril like with one or two spirals

F Perstans

♀ 1 Length seventy to eighty mm

2 Neck longer than *F Bancrofti*

3 Body without markings

4 Tail incurvated Tip of tail mitred This is *characteristic* of this species

5 Mouth minute simple

6 Embryos in utero blunt tailed not sheathed

♂ 1 Length forty-five mm

2 Head end as in female

3 Two caudal ends much coiled

4 One spicule and two papillae

5 Low describes four pairs of pre anal and one pair of post anal minute papillae

F Ozzardi —Adult

1 Dimensions much the same as those of *F Bancrofti*

2 Distinguished by the *bulbous* tail in *F Bancrofti* it is not bulbous but circular

F loa —Adult forms travel about in connective tissue

♀ 1 Length thirty to forty mm (average) Varies from sixteen to seventy mm Breadth 0 57 mm

2 No neck Head cone shaped

3 Body cylindrical tapers sharply towards head and tail

4 Cuticle with bosses except over the head

5 Tail terminates in a short incurved portion and has two small tubercles at its extremity

6 Mouth simple

7 Ova containing embryos thirty-five μ by twenty-five μ

8 Anal orifice on low broad papillae 0 3 mm from the tip

♂ 1 Length twenty five to thirty mm

2 Uniform thickness except at head and tail

3 Cuticle with bosses but not so numerous as in the female

4 Tail not spirally twisted merely incurved It possesses well-marked lateral alae

5 Three well marked pre-anal papillae and two unequal post-anal papillae Two slender unequal spicules

F Megalhaesi —Adult males and females in left ventricle

♀ 1 Length, one hundred and fifty-five mm by 0 7 mm

2 Club shaped oral end

3 Swollen oesophagus well marked

4 Mouth simple

5 Cuticle fine striations

♂ 1 Length eighty-three mm by 0 4 mm

2 Four pairs pre-anal four post-anal papillae and two spicules

To Examine Blood for Filaria Embryos

The technique varies somewhat according to what end the observer has in view

1 To facilitate detection it is well as MANSON advises to make thick films of blood Dry Then wash out the haemoglobin with water or one third per cent acetic acid and stain with haematin or gentian violet or fuschin

For the latter stains a few drops of a saturated alcoholic solution of the dye are added to half a watch-glass full of water

In these methods no fixation has taken place and the parasites are exposed directly to the action of a watery die

2 (For studying the minute structure of the embryos the above method is not advisable) Make a film in the ordinary way Fix in alcohol and stain with haematin The spots and granules of the embryo are most beautifully shewn

Filaria in Mammals

F. immitis.—Adults found in right ventricle of dog, fox, and wolf. Embryos in blood. Embryos develop in the malpighian tubes of *Anopheles*. Afterwards they enter the general body cavity and pass towards the labium.

Dogs in the tropics commonly harbour this filaria. The filariae are most numerous at night.

F. recondita.—A single female adult has been found in kidney of dog.

Embryos in Blood.—Embryos develop in *P. serraticeps* (dog flea) and *P. irritans* (man and dog), also in a dog tick. They are found in the intestine and body cavity. The filaria has, however, not been transmitted from infected fleas to healthy dogs.

F. equina.—Serous cavities, intestines, and liver of horses, donkeys, and mules.

Embryos in Blood.—Appear like *F. Bancrofti* but smaller.

F. haemorrhagica.—Male and female live together in tissues of horse and donkey. They form hemispherical tumours the size of a nut beneath the skin. These burst and discharge blood. Fresh tumours appear in from one to two days.

F. irritans.—Found in summer sores of horses and donkeys.

F. Evansi.—Lung and mesentery of camel. Embryos in blood.

F. lacrymalis and *F. palpebralis*.—About the eyes of horses and cattle.

F. osleri.—The adults cause broncho-pneumonia in dogs.

5 Three well marked pre-anal papillae and two unequal post-anal papillae Two slender unequal spicules

F Megalhaesi — Adult males and females in left ventricle

♀ 1 Length, one hundred and fifty five mm by 0 7 mm

2 Club shaped oral end

3 Swollen oesophagus well marked

4 Mouth simple

5 Cuticle fine striations

♂ 1 Length eighty-three mm by 0 4 mm

2 Four pairs pre-anal four post anal papillae and two spicules

To Examine Blood for Filaria Embryos

The technique varies somewhat according to what end the observer has in view

1 To facilitate detection it is well as MANSON advises to make thick films of blood Dry Then wash out the haemoglobin with water or one-third per cent acetic acid and stain with haematin or gentian violet, or fuschin

For the latter stains a few drops of a saturated alcoholic solution of the dye are added to *half a watch-glass full of water*

In these methods no fixation has taken place and the parasites are exposed directly to the action of a watery dye

2 (For studying the minute structure of the embryos the above method is not advisable) Make a film in the ordinary way Fix in alcohol and stain with haematin The spots and granules of the embryo are most beautifully shewn

Filaria in Mammals

F. immitis.—Adults found in right ventricle of dog, fox, and wolf. Embryos in blood. Embryos develop in the malpighian tubes of *Anopheles*. Afterwards they enter the general body cavity and pass towards the labium.

Dogs in the tropics commonly harbour this filaria. The filariae are most numerous at night.

F. recondita.—A single female adult has been found in kidney of dog.

Embryos in Blood.—Embryos develop in *P. serraticeps* (dog flea) and *P. irritans* (man and dog), also in a dog tick. They are found in the intestine and body cavity. The filaria has, however, not been transmitted from infected fleas to healthy dogs.

F. equina.—Serous cavities, intestines, and liver of horses, donkeys, and mules.

Embryos in Blood.—Appear like *F. Bancrofti* but smaller.

F. haemorrhagica.—Male and female live together in tissues of horse and donkey. They form hemispherical tumours the size of a nut beneath the skin. These burst and discharge blood. Fresh tumours appear in from one to two days.

F. irritans.—Found in summer sores of horses and donkeys.

F. Evansi.—Lung and mesentery of camel. Embryos in blood.

F. lacrymalis and *F. palpebralis.*—About the eyes of horses and cattle.

F. osleri.—The adults cause broncho-pneumonia in dogs.

Avian Filariae

Filarial embryos are very common in the blood of birds, and the adult forms are found in the most diverse positions, notably in the sub cutaneous tissues In some form the embryos appear to be confined to the lymph

In the description of *Avian filaria* the following should be noted —

1 The species of bird concerned

2 The site of the adult filariae

3 The description of the adult filariae female and male the use of COBB'S formula gives uniformity to descriptions The measurements are taken with the animal in profile from the anterior end

- (i) To the base of the oesophagus
- (ii) To the nerve ring
- (iii) To the cardiac constriction
- (iv) To the vulva in the female, or to the middle in male
- (v) To the anus, noting when this is terminal

At each of these points transverse measurements are taken and noted below the above, so

Longitudinal
Transverse

The unit of measurement is one hundreth part of the length of the worm

This formula should be used with caution, since it rests on the assumption that the proportions of the various parts of the body are constant in different individuals (SHIPLEY)

Drawings should be made of the head and tail and the mouth anus and vaginal orifice carefully described

4 The description of the embryo Where found blood or lymph Presence of a sheath Length and breadth of embryo and sheath The exact description of spots and the distance of these from the anterior extremity The following spots and markings may be seen –

(i) A transverse slit about twenty five per cent length Sometimes not seen

(ii) A clear, sometimes lateral sometimes transverse spot about thirty to forty per cent length

(iii) A long space in which the nuclei are loosely arranged often ending anteriorly and posteriorly in clear space About sixty five per cent length

(iv) A small spot about seventy-six per cent length

(v) Very small lateral spot or slit ninety per cent length

Development of Filaria in the Mosquito

Experiments made so far have been chiefly with *F nocturna* (*F Bancrofti*)

Both *Anopheles* and *Culex* mosquitoes may act as the hosts of *F Bancrofti* Certain species of both genera however do not act as hosts The following have been shewn to act as hosts —

C pipiens	*P Costalis*
C ciliaris	*Myz. Rossii*
C fatigans	*My. Sinensis*

The following have been shewn by BANCROFT not to allow full development to take place. In some species partial development occurs, the larva, however, eventually disappearing:—

C. notoscriptus (SKUSE)
C. annulirostris „
C. hispidosus „
C. vigilax „
C. Nigrothorax (MACQUART)
C. procax (SKUSE)
[*A.*] *Musivus* „

GRASSI and NOE'S experiments shew that *F. immitis* is capable of developing in *A. claviger*. As regards the re-infection of a healthy dog, the experiments are somewhat inconclusive, for in the dog used a single immature worm only was found, about sixteen days after the period of biting. They state, however, that of a batch of *Anopheles* dissected before the 'biting,' many of the labia contained filariæ, whereas, of a batch dissected after the 'biting,' none contained filariæ, the conclusion being that the filariæ had escaped through the labia into the blood of the dog during the 'biting.'

Seven stages of development of the embryo are usually described. The following is a resumé of the changes undergone in *Culex pipiens*:—

First Stage.—One hour after removal of blood by mosquito the sheath is cast and the embryos exhibit active locomotive movements. In twelve to eighteen hours many have bored through the stomach wall and have reached the

muscles Some die within the stomach In the muscles the cuticular striation disappears movement ceases and the body becomes thicker

Second Stage —For two to three days the embryo becomes much thicker and the mouth begins to be faintly indicated

Third Stage — An anus appears in front of the tail and a mouth is very distinct with four fleshy lips Cells are seen in the body and these form an alimentary and tegumentary layer The embryo is now about 0 3 mm long

Fourth Stage —Rapid growth takes place and the tail becomes relatively smaller

Fifth Stage —Lengthening takes place The whole worm becomes fibrous and transparent in appearance It has cast the cuticle Some large cells at the end of the tail form papillae which are characteristic of this stage of the larva The parasite is now about 1 5 mm (one-sixteenth inch) Time about seventh day

Sixth and Seventh Stages —Movements become more active and when the filariae have reached their highest stage of development in the thoracic muscles they leave that tissue and travel forward in the direction of the head of the mosquito (Low and James) They reach the loose tissue about the salivary glands and pass into the neck Some are found in the abdomen Numbers of the filaria larvae enter the lower part of the head lying beneath the large head ganglia Eventually one or more worms pass into the substance of the labium where they are readily found by dissection The larva at this stage measures about one-sixteenth inch in length

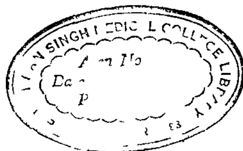
The Transmission to Man

According to Dutton, who has very minutely described the structure of the proboscis, the worms can only leave the labium at one point, i e, by perforating an extremely delicate membrane, which closes in the extreme end of the labium (see p 166) If they escape elsewhere they must penetrate the dense and hard chitinous envelope of the labium—a very improbable occurrence

LITERATURE

Railliet A *Traite de zoologie medicale et agricole* Paris 1895 2nd edition

Annett Dutton and Elliott *Liverpool School of Tropical Medicine* Memoir IV Part II *Filariasis*



APPENDIX

APPENDIX

BLOOD SUCKING FLIES

The Diptera or flies are two winged insects (the posterior pair of wings are transformed into *halteres*) and are so distinguished for example from the Hemiptera or bugs which generally have four wings. In the Diptera the metamorphosis is complete eggs larva pupa insect in the Hemiptera it is not so. The following classes have blood sucking habits

The Nematocera (*nema* thread *keras* antenna) including the following families —

1 —*Blepharoceridae*
Wing iridescent no discal cell on wing (the discal cell lies between the second posterior cell and the basal cell) They resemble midges. The larvae have suckers and are found attached to stones in the water
Genus *Curupira*

2 —*Culicidae* Mosquitoes or gnats

3 —*Chironomidae* (Midges)
[Genus *Chironomus*] not blood sucking. Larvae are blood worms. Very diverse
Genus *Ceratopogon* Very minute midges. Wings generally spotted. Larvae mostly non aquatic
C varius A pest in Scotland

4 —*Psychodida* (Moth flies)
Very small. Antennae very hairy. Wings very hairy. Larvae of some genera amphibious
Genus *Phlebotomus* S Eur pest

5 —*Simulidae* (Sandflies Buffalo gnats)
Small humpbacked flies. Antennae destitute of hairs. Wings relatively large. Proboscis short thick consisting of epipharynx and hypopharynx. Antenna eleven segments. Palpi four segments. Larva aquatic
Genus *Simulium*

The *Brachycera* ($\beta\rho\alpha\chi\upsilon\varsigma$ short $\kappa\epsilon\rho\alpha\varsigma$ antenna) include the following —

1 —*Tabanidae* (Horse flies or gad (=sting) flies)

Large flies Antenna three jointed not terminating in a style or arista (the arista (when bristle like) or style (when thick) being an appendage of the terminal portion (flagellum) of the antenna) Labium enclosing four stylets in ♂ six in ♀ The terminal joint of the palpi is inflated and the palpi hang down in front of the proboscis

(*a*) Genus *Tabanus*

Proboscis short and thick vertical in the female oblique in the male terminal joint of antenna crescentic Flight humming Attacks horses cattle man

(*b*) Genus *Haematapota*

Terminal joint of antenna not crescentic Wings adjacent like the sides of a roof No ocelli Flight silent

H pluvialis Common in woody lanes in England in the summer

(*c*) Genus *Pangonia*

Proboscis often long thin horizontal In some three to four times length of body piercing even when the fly is on the wing Accessory eyes (ocelli) generally present Hind tibiae spurred The Seroot fly of Nubia is probably a species of Pangonia Whether the Zimb fly of the Arabs or the Tsaltsallya is the same is doubtful

(*d*) Genus *Chrysops*

Three ocelli Second joint of antenna as long as first Eyes golden green Flight silent Wings widely separated Spotted Hind tibiae spurred

Ch caecutiens attacks the eyes especially

(*e*) Genus *Hadrus*

H lepidotus = Motuca fly of Brazil

The third group (*Cyclorrhapha Schizophora*) include the muscinae sarcophagidae and oestridae The last two groups are not blood suckers but are included here for their pathological interest also only some of the first group are blood sucking

1 —*Muscinae*

Antennae have three joints and an arista wing veins less complex than in *Brachycera*

(*a*) Genus *Musca* House flies (not blood suckers)

(b) C nus *Calliphora* Blow flies or blue bottles (not blood suckers)

(c) Cenus *Lucilia* Gr en b ttles

L macellaria The larv of th fly is the Americ an scr w w rm infesting the nasal fossae and frontal sinuses of man

(d) C nus *Stomoxys*

Pr boscis horizont il l ng and firm Third segment of antenna three times as l ng a s nd Arista feathered n it upper surface only Palpi n t l ng r than the epi t me

S Calcitrans r semble th h u e fly but it head is raised Attacks cattle c asionally man Attempts to trans mit *T Brucei* with this fly have been negative

(e) Cenus *Haemat bia*

Small flies Palpi as l ng as probo ci arista f ather d above and partly below Attack cattle fi r ely

(f) C nu Glossina

Arista feath red above *L de a tea*

—*(Sarcoph igidae)*

Not blood suckl ng Arista feathers at the base bare at th tip Large flies ab ut 14 millim tr s l ng

C nus *Sarcoph iga* 1 l ng te l th ax three bla k band abdomen p tted Third s gment l nut n t three times th second segment

S carnaria S magnific a and S l ip im (India) g v e rise to terrible forms of myia is in man and animal

3—*(O stri lae)* (Bot (=B ury) Flie)

Not blood su king Large flies Pr bosc is rudimentary Antenna very sh rt Arista egmented Flight humming

(a) Cenus *Ga t ophil is e g G equi* The white eggs can be e sily seen on the horse s hair The larva are sw ll owed and they attach themselv s t th mu osa f the st ma h

(b) Cenus *Hypoderma e g H lin ata* Larvae produce ox warbles (=tumours) in the x

(c) Cenus *Oestrus e g O Ovi* Larvae in the respirat ry passag s cf the she p

(d) Cenus *Cephal myia e g C m ulata* In the camel

(e) Cenus *Ceph n myia e C auff rdi* In r d d er Scotland

(f) Cenus *Dermatobia e g D noxiaventris* Larva in the ver macaque (America) producing myia is in man and cattle

(*g*) Genus *Ochromyia* e g *O anthropophaga* Larva is the ver de Cayor (Senegal) producing myiasis in man

Myiasis is common in Africa and in the tropics but the larvae have been identified in but few instances as yet

The fourth group the Pupipara (to which *Glossina* also belongs from the point of view of its life history) comprises

1 —*Hippoboscidae*

Labium absent Proboscis consisting of epipharynx and hypopharynx ensheathed by the maxillae Palpi wanting Deposit larvae which subsequently become pupae They suck the blood of mammals and birds Their wings are often very minute

(*a*) Genus *Hippobosca* (spider flies)

Wings large obtuse No ocelli arista nude legs long and extended

H equini Runs rapidly over the body is the [New] forest fly of England

H camelina Attacks camels in Egypt

H rufipes transmits *Trypanosoma theileri*

(*b*) Genus *Melophagus*

Wings extremely minute No arista on antennae

M ovis is the sheep tick Four millimetres long

(*c*) Genus *Ornithomyia*

Wings large Four millimetres long

O avicularia Occurs on birds

(*d*) Genus *Lipoptera* e g *L cervi* on the red deer

(*e*) Genus *Stenopteryx* e g *S hirundinis* of the swallow

—*Nycteribiidae*

Found on bats They have no wings

FLEAS †

Fleas or Aphaniptera are considered to be aberrant forms of flies and hence follow naturally after the division Pupipara of the flies

† [illegible]

Life History

Eggs About a dozen are laid in floors in cracks etc sometimes in the hair or fur of animal They are 0 7 by 0 4 millim tres (*P irritans*) The eggs hatch in about a week or more

Larvae Are worm lil whitish consisting of fourteen segments They are about one and a half by one tenth millimetres in size They feed on organic refuse (?) n blood In about eleven days th y are full grown They wrap themselves up in a co o m m ult an l b com

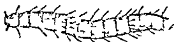


Fig 90 Larva of a Fl a (F lu num) x o
(*After* Raillieti)

Nymphs—Afte the laps of an ther eleven day th fl a emerges (the evolution thus taking about a month)

Adults—The female ha a n ave back the mal a convex back

Anatomy

1 The head is small not distin tly eparated fr m th body

The antenna are placed in fos a behind the eyes They consi t cf two basal egm nts and a third of div r f rm irr gularly s gm nted Th maxillary palpi must not be mistaken f r them

3 He m uth c nsist cf (a) epipharynx (the central styl t) s rr ited above tubular bel w (b) tw err t l man dibles hollowed on their inner urf es and f rming with (a) a gutter along which th bl od fl w ( ) a l bium ingle f r a sh rt di tance then bifurcating and forming t v labial palp wh h f rm a heath f r the pi r ing organ (a) and (f) (f) two maxillae having th f rm f expand d plates e ch bearing a f ur jointed palpus

4 The thoracic egment thre in number ar separate

5 Abdomen n i ting cf nine gm nt overl pp in th dorsal and ventral portions n t bein unit d and s allowing cf di ten i n

6 Spines arranged in comb like fashion exist on the lower part of the head pro and meta thorax and abdomen These are of great importance in classification

The *Aphaniptera* comprise (1) the *Pulicinae* (fleas proper) (2) the *Sarcopsyllinae*

The *Pulicinae* have—

1 Small head
2 Labial palps four joints
3 They are never stationary parasites

There are three genera (1) *Pulex* ( ) *Hystrichopsylla* (3) *Typhlopsylla*

Genus *Pulex*

1 Well developed eyes
2 The eggs are not fixed to hairs etc

P irritans

1 No spines on lower portion of head
No spines on pro thorax
3 Third joint of antennae incised
4 Parasitic on man



Fig 91 *P irritans* x 20 (*After* Railliet)

P serraticeps

1 A comb of seven to nine spines on each side of the head
2 Pro thorax dorsally a similar comb
3 Parasitic on dog (? same species on cats)

P goniocephalus

1 Head bent into an obtuse angle Above and laterally has combs of five to six spines
Pro thorax posteriorly six long narrow spines
3 Parasitic on rabbits

I avium

1 Head no spin s
2 I rothorax twelve to thirteen posteriorly
3 I arasitic on birds Doubtful if all are of the same species

There are a large number of other species

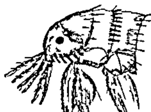


Fig 9 *P Serr aticeps* x 30 (*Afte* RAILLIET)

Genus *Hystrichopsylla*

1 No eyes
Head truncated

A single spec s xi t n the mole an l v le

Genus *Typhlopsylla*

1 Eyes rudimentary or absent
Head elongated rounded in front

The spe ies are di tingui he l by th number f th ir spine combs *e g I ct itenus hex t un p nt tenus* t They are parasitic on bat mole hr w mi rat le fi ld mice

The *Sar opsyllinae*

1 He d l rg
Thorax narr w
3 The t rmin l j int f th ant nna i n t egment l

Comprise thr e genera (1) *S r psyll* (2) *Rhyn p ylla* (3) *Vermipsylla*

Genus *Saro psylla*

1 Head angular
Eyes small
3 Maxillae mall

4 Epipharynx elongated
5 The abdomen in the female capable of great distension They imbed themselves in the tissues

S penetrans (CHIGGER) On man pig horse goats and even it is said birds

S gallinarum On the eyelids and necks of fowls (Ceylon)

Genus *Rhyncopsylla*

1 Head rounded
Maxillae curved

A single species found on parrots and bats Are fixed like ticks with the body free

Genus *Vermipsylla*

1 Head rounded
2 Maxillae large and triangular

V Alakurt Parasitic on horses (Turkestan) Fixed with the body free

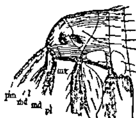


Fig 93 *P Goniocephalus* x 30 (*After* RAILLIET)

LITERATURE

Camb dg N t l H to y *I t* Pt II
R ll t *T ed Zool g m l l t g ol*

STAINS NUMERICAL DATA ETC

FIXING AND HARDENING SOLUTIONS

Alcohol is a fixative and dehydrating medium and for ordinary work is the mo t convenient The tissues in small pieces may be placed directly in methylated spirit (ninety per cent alcohol) or absolute alcohol (ninety eight per cent alcohol) Change the alcohol a few times *Or pass through*

fifty seventy five ninety five one hundred per cent alcohol leaving a few hours in each After hardening of the specimen are not to be imbedded immediately tran fer to alcohol of about eighty per cent for preserving After the use of other fixatives specimens should be washed and transferred to eighty per cent of alcohol for preservation

Rectified spirit of the British Pharmacopoeia is equal to eighty four per cent alcohol

Methylated spirit containing wood naptha is equal to ninety per cent alcohol

Ordinary methylated spirit contains mineral naphtha and should not be used

Absolute alcohol is equal to ninety eight per cent alcohol For practical purposes the dilution of alcohol is sufficiently accurately made by means of the diluting formula (p xxii)

Zenker's Fluid —

Potassium bichromate	5 gramme
Sodium Sulphate	1 0 gramme
Corrosive Sublimate	5 0 gramm
Water	100 gramm

Add glacial acetic acid to this stock solution in the proportion of five gramme to one hundred before use Fixation is complete in one to twenty four hours Wash thoroughly in alcohol to which enough iodine has been added to give a dark brown solution Or if al h l a mixture of iodine two part potassium iodide one part glycerine fifty part water fifty part Renew until no further decolorisation takes place

Haematin or e in and methylene blue give good results for material thus

Orth's Fluid—Th Muller fluid to which formalin (i e formaldehyde forty per cent has been added in the proportion of ten c to one hundred of Muller before use

Fixation of small pieces takes place in two to three hours if kept warm Wash thoroughly in running water

Muller's Fluid —Potassium Bichromate		2½ part
	Sodium Sulphate	1 part
	Water	100 part

Add a little camphor or naphthalin to prevent the growth of mould Change the fluid after twenty four hours and then every few days for the first week Tissues are ready in a fortnight or three weeks They may be left much longer Fix

in the dark. Wash thoroughly in water till colourless. Transfer to alcohol seventy to eighty per cent. for preservation.

Flemming's Solution.—Chromic acid 1 per cent. 15 vols.
Osmic acid 2 per cent. 4 vols.
Glacial acetic acid 1 vol.

Mix in the above proportions before use. Use very small pieces. Fixation is complete in about twenty-four hours. Blackening due to the osmic may be removed by hydrogen peroxide. *Blood films may be fixed in this solution.*

Tissues thus fixed may be preserved in equal parts of alcohol and glycerine.

Formalin (forty per cent. solution of formaldehyde).—Use two to five per cent. solution in water. Small pieces are fixed in twelve to twenty-four hours. They may be left in solution or transferred to alcohol.

Corrosive Sublimate.—Best used as a concentrated alcoholic solution (or aqueous may be used). Fixation takes place in a few hours. Wash thoroughly in water and transfer to iodine solution (*vide* Zenker's fluid) till iodine no longer decolourized.

The concentrated alcoholic solution is a most rapid fixing and hardening reagent, and sections can be cut in a very short time if small pieces are used.

Decalcifying Solution.—Tissues require fixing before and after these solutions.—

(i) Phloroglucin one gramme, nitric acid ten c.c., water *one hundred c.c.*, *or*

(ii) One to five per cent. solution of nitric acid in water or alcohol. Change the fluid daily. Decalcification takes place in two to three days.

(iii) Picric acid, a saturated solution (= about 0.75 per cent.) containing crystals. Decalcification may take weeks or *months. Wash in alcohol.*

Eau de Javelle (dissociating and decolourizing solution).—Add to a concentrated aqueous solution of chloride of lime a solution of potassium oxalate as long as a precipitate is formed. Filter and dilute if necessary. This may be used for softening the chitinous skeleton of mosquitoes and for decolourizing Madura fungus, etc.

For Fixing Paraffin Sections to the Slide

1. Celloidin one part, oil of cloves two parts, *or*
2. Thin solution of white shellac in creosote, *or*

3 Thoroughly mix equal part of white of egg and glycerine filter This is one of the simplest and best mean or

4 Filtered white of egg 50 c c
Glycerine 50 c
Sodium salicylate 1 gramme
Shake well and filter (this takes about a week) The solution keeps well for six months or more or

5 Simply use fresh white of egg smear thinly over dry

MOUNTING MEDIA ETC

1 *Farrant's Solution*—

i Take equal parts of glycerine and a saturated solution of arsenious acid Add powdered gum arabic till the solution is saturated or

ii Pure gum arabic 40 gramme
Water 40 c
Glycerine 0
Carbolic acid 1 gramme
[or Thymol 0 3 gramm]
Powder the gum and dissolve in about one hundred and fifty c.c. of water by boiling add the carbolic acid ... a little water filter through a hot filter changing when clogged evaporate until it is about ... then add the glycerine

Acetate of Potassium—Saturated solution Cement the cover glass with gold size ... This is used for osmic preparations and for ...

Glycerine Jelly—Glycerine
Water
Gelatine 1 gramme
Thymol 0 5 gramm
or one gramme of phenol to each one hundred grammes of the mixture

Dissolve the gelatine at forty degrees in water but add the glycerine (warmed) ... little water and stir in ... stir continuously ... warm to eighty five degrees filter through a hot filter

TO MOUNT DELICATE OBJECTS

Place in ten per cent glycerine ... allow this to concentrate in the air and then transfer to the jelly

To mount in Jelly —Remove as much water as possible place the slide coverglass and jelly in the incubator (if necessary) Before cementing see that the layer of jelly is not too thick If too thick press some out and scrape away then cool

Dammar Lac —Dissolve in equal parts of benzene and oil of turpentine It does not render preparations as translucent as Canada balsam

Fixing Blood

As we have already stated for practical purposes alcohol is absolutely satisfactory *The following solutions have been* used and may prove useful occasionally —

1 *Osmic acid* —Osmic acid 1 0 sodium chloride 0 6 distilled water 1 0

A neat and practical method of using this is to moisten a camel's hair brush with the solution then to touch the blood drop and to immediately spread the blood out on the slide with the brush Wash the brush after use in alcohol (Kornilowitch)

2 *Chloroform* —Instead of heat in staining with Ehrlich's triacid Fix for five minutes in chloroform (neutral to litmus paper) Stain for five minutes or more after fixing (Josué)

3 *Strong Flemming* Especially for nuclear structures of parasites

4 *Heat* —Heat up to one hundred and fifteen to one hundred and twenty degrees C in a hot oven and then when this temperature is reached allow to cool again in the oven

5 Osmic acid two per cent glacial acetic acid equal parts Expose to the vapour For delicate work indispensable

Staining Solutions for Blood etc

1 *Romanowsky stain* (p 10)

Haematin (p 50) —If the solution has become reddish on keeping neutralize with a little ammonia

3 *Eosin and Methylene Blue* (consecutively) —(a) Stain for one to five minutes in a one half per cent solution of eosin in sixty per cent alcohol wash dry with blotting paper and stain (b) in a saturated watery solution of methylene blue for thirty seconds to one minute

This is a useful and simple method for studying the acidophil and basophil reactions of granules

4. *Safranin.*—(*a*) Safranin O (soluble in water). The solution must be heated up to sixty to eighty degrees C., and subsequently filtered. (*b*) A saturated alcoholic solution of safranin (soluble in alcohol). Take (*a*) and (*b*) *partes aequales*. Stain for five to ten minutes or less.

5. *Ehrlich's Triacid.*

6. *Ehrlich's Haematoxylin Eosin.*—Haematoxylin, [illegible] grammes; acetic acid, twenty grammes; alcohol, one hundred grammes; glycerine, one hundred grammes; water, one hundred grammes. Allow this to ripen for a month in the sun, then add eosin to the extent of one per cent.; stain for twenty-four hours. The solution is best got ready made.

SOLUBILITY OF STAINS

10 c.c. of saturated alcoholic methylene blue contains [illegible] grammes of the stain.

10 c.c. of saturated aqueous methylene blue contains [illegible] grammes of the stain.

10 c.c. of saturated alcoholic gentian violet contains [illegible] grammes of the stain.

10 c.c. of saturated aqueous gentian violet contains 0·175 grammes of the stain.

10 c.c. of saturated alcoholic fuchsin (basic) contains [illegible] grammes of the stain.

10 c.c. of saturated aqueous fuchsin (basic) contains [illegible] grammes of the stain (Hewlett).

Löffler's Methylene Blue.—[illegible] saturated alcoholic [illegible] methylene blue [illegible]

100 c.c. [illegible]

Ordinary Methylene Blue.—A saturated alcoholic solution is used as the stock, and a few drops added to a watch-glassful of water according to strength required, or ten per cent. solution is a convenient strength.

Borax Methylene Blue ([illegible]).—Saturated aqueous solution of methylene blue, twenty-four parts; borax five per cent. solution, sixteen parts; water, forty parts.

The times necessary for staining are best judged by the appearance of the films or tissues.

TISSUE STAINS

1. *Haematein* ([illegible]).—Stain for [illegible] minutes according to the [illegible] of the solution. [illegible] readily overstain. Decolorize, if necessary, with [illegible] per cent.

alum solution Counterstain if required with a weak watery solution of eosin one half to one per cent or the sections may be stained first with a strong eosin solution five to one hundred per cent for five to twenty minutes Combination of eosin and methylene blue can be used in a similar way

2 *Alum Carmine*—Carmine two grammes alum five grammes water one hundred c c Boil together Filter Does not overstain

3 *Ehrlich Biondi*—Saturated aqueous solution of rubin s four parts orange g seven parts methylene green eight parts Dilute fifty to one hundred times before using Stain for twenty four hours Wash in alcohol The sections may before staining be treated with acetic acid (two parts in one thousand of water) for a few hours

Iron Reaction (Haemosiderin) in Malarial Tissues

1 Fix in alcohol

2 Two per cent aqueous solution of potassium ferro cyanide five to twenty minutes

3 Acid alcohol (HCl one part seventy per cent alcohol, one hundred parts) five to ten minutes

4 Wash in water

5 Counterstain with alum carmine

Staining of Amoeba Coli in Tissues (Mallory and Wright)

1 Fix in alcohol

Saturated aqueous solutions of thionin three to five minutes

3 Two per cent solution of oxalic acid one half to one minute

4 Wash in water

5 Dehydrate in alcohol

6 Clean in oleum origanum cretici

7 Wash in xylol

8 Balsam

Nuclei of the amoebae are brownish red the nuclei of the mastzellen are blue

Weights and Measures etc

1 Conversion from one temperature scale to another—

$$\frac{C}{5} = \frac{R}{4} = \frac{F-3}{9}$$

Thus to convert 100 F to centigrade—

$$\frac{100 - 3}{9} = \frac{C}{5} \quad C = 37\ 7$$

Formula for dilution of Solutions.—The number of parts required to dilute from one part of a solution of strength x per cent to another strength y per cent is $\frac{x}{y} - 1$

Thus to dilute a solution from 25 per cent to 5 per cent add to each volume of solution $\frac{20}{5} - 1 = 3$ of the diluting fluid

3 *Approximate Values*.—

1 cubic centimetre = 17 minims 1 minim = 059
100 = 3 ounces 4 drachms 0 minims
1 drachm = 3 55 c
1000 cubic centimetre = 1 76 pints 1 fluid ounce = 28 4 c c
4 litres = 7 pints 1 pint = 568 c c
1 gramme = 15 4 grains (avoirdupois) 1 grain (avoirdupois) = 0 648 grammes
1 kilogramme = pound 3 ounces 11 5 grains (avoirdupois) 1 drachm = 1 77 grammes
5 kilogrammes = 11 pounds 1 oz = 28 35 gramme
1 grain apothecaries = 0 648 gramme
1 drachm apothecaries = 3 88 grammes
1 ounce apothecaries ℥ = 31 1 gramme
15 4 grains apothecaries = 1 gramme

4 1 millimetre = 039 inches 1 inch = centimetre
1 centimetre = 39 inches 1 inch = 25 33 millimetre
1 metre = 3 8 feet inch = 1 millimetre
the millimetre (μ) = 0004 inches
5μ = inch μ = inches

5 π = 3 14159
Circumference of a circle = $2\pi r = \pi d$
Area of circle = πr^2

LIST OF APPARATUS

		£	s	d
1	A Beck microscope including oil immersion	13	1	0
2	Glass slide 3 x 1 ground edges per gross	0	3	6

[illegible footnote]

		£	s	d
3	Cover glasses No 1 ¾ in ⅛ oz	0	4	0
4	Straight surgical needles for making blood films and dissecting ½ doz (Weiss & Co Oxford Street)	0	1	6
5	Stoppered jars (4) 13 x 7½ centimetres	0	3	6
6	Porcelain dishes square flat 1 doz	0	1	0
7	Specimen tubes flat bottomed corked 3 in x ¾ in 50	0	4	0
8	Slide box to hold 25 sliding cardboard	0	1	0
9	Measures 100 c c and 10 c c	0	1	9
10	Drop bottle for xylol (a toothpick inserted into the cork of a specimen tube serves the purpose)	0	0	4
11	Cedarwood oil bottle (a pin in the cork of a specimen tube makes a convenient *dropper for the oil*)	0	0	9
12	A Primus paraffin burner for boiling etc	0	12	6

STAINS ETC

1	Romanowsky —Methylene blue pure medical 10 grammes or in soloids	0	1	8
	Eosin B A 10 grammes or in soloids	0	1	
	Sodium carbonate pure 1 oz	0	0	
2	Leishmann's Stain—(*a*) In soloids (Burroughs Wellcome & Co) = 0 15 gramme Dissolve in 10 c c of methyl alcohol (or methylated spirit)			
3	Haematin 5 grammes	0	4	6
	Alum 1 oz	0	0	-
4	Absolute alcohol 1 lb	0	4	0
5	Xylol 1 lb	0	2	3
6	Paraffin wax melting points 50 C and 60 C 1 lb 2s 6d 1 lb 3s	0	5	6

ADDITIONAL APPARATUS

1	A mechanical stage fitting on to *the stage* (not the column) of the microscope	3	5	0
	Browning's pocket spectroscope indispensable for urine work in blackwater fever etc.	1	0	0

FOR MOSQUITO COLLECTION

		£	s	d
1	A lens (the lens of an eye piece of the microscope serves a well)			
2	Silver pins No 20 ¼ oz	0		0
3	Entomological pins 1½ in long 1 oz	0	0	8
4	Cardboard (fine Bristol board) 4 sheet	0	0	3
5	Specimen tubes flat corked each about	0	0	1
6	Pill boxes			
7	A dissecting board 10 x 3 in half covered with black half with white paper (made by self)			

Supplied by Messrs C Baker & Co London

INDEX

A

Abdomen of larvae 33
Abdomen of mo quitoe 171
Acartomyia 173
Achromaticus vesp rug inis 3 1
Aedeomyina 1 4
Aedeomyia 151
Aedes 150
Aedimorphus 181
Aestivation of *Anophelina* 17
Africa *Anophelina* of 08
African coast fever 331
Africa quartan para ite in 68
Aino 360
Albuminuria in malaria 283
Alcohol a fixative X
Alcohol to dehydrate 47
Aldrichia genus 186 96
Al error 06
Alum carmine XVI
Amblyomma 340
America *Anophelina* of 08
Amoeba Coli staining f XVI
Ammonia as larvicide 53
Amplugens 56
Anopheles attitude of 63
genu 185 190
larva 74 77 1
nympha 88
peci of 131 13
the male 18
un pott d wings 6
A aitkenii 13
algeriensis 131
bifurcatus 131
cru ians 131
gigas 131
A immaculatus 13
lindesayii 131
ma ulip nnis 131
nigripes 13
philippinensis 19
pseudo pun tip nni 131
pun tip nnis 131
stigmaticus 13
rugus 145
walkeri 131
Anophelina 173
aestivation of 217
breeding pla e of 33
chara ters of 6
cla sification f 184
di persal of 1
d me tic 213
eggs 6
f undati n f 18
fe d f 14
flight f 215
ge graphical di
tributi n 07
hiberati n f 11
larva of 2 8
length f life f 17
Anophelina f Africa 08
Ameri a 08
Au tralia 09
Europe 08
India 09
Malay ia 09
I le tine 08
Anophelina ova f 220
palpi f 6
post scutellum
f 168

Anophelina relation to colour of 16
salivary glands of 119
scales of 186
seasonal prevalence of 10
species differentiation of 188
wing of 169
wild 15
Antennae arista of 350
of larva 8- 2 9
of mosquitoes 164
Aphaniptera VI
Aponomma 339
Apparatu list of XVII
Argas 341
Argasidae 335 340
Arista of antenna 350
Armigeres 177
Arribalzagia genus 185 199
maculipes 200
Artificial appearanc s 14
Australia *Anophelina* of 09
Avian *filariae* 374

B

Basophil staining 6
Bats parasites in 3 0
Beccarimyia 351
Bile pigments in urine 255
Bilirubin in urine 259
Bird feeding experiments on 10
filariae in 374
parasites in 314
to feed mosquitoes on 101
Black spores 317
Blackwater fever and quinine 303
blood in -1 309
Blackwater fever kidn ys in 31
leucocytes in 308
Methaemoglobin in 85
parasites in 308
pigment in 305
post mortem changes 311
urine in 310
urobilinuria in 311
Blepharoceridae III
Blood anaemic 5
dust 0
films dry to prepare seq
films to examine 3
films to fix 8 seq
films to label 6 seq
films to stain 9
films wet to prepare 5
fixatives for XIV
guaiacum test for 85
Heller s test for 85
in blackwater fever 1 309
isotonic point of 81
normal constituents 17
spectroscopic test for 85
sucking flies III
to examine for *Filaria* 37
to examine for *Trypanosomes* 36
Boophilus 338
Borax methylene blue XV
Bot flies V
Brachiomyia 180
Brachycera IV
Breeding out of mosquitoes 157

Breeding places in dry
season 1
of Anopheline 3
Buffalo gnat III

C

Callithyra X
Cannibal larva 81 83
Carmine alum XVI
Cecid myidae 5
Cellia genus 1 6 5
species 5
allipes 8
argyrotarsis
kochii 8
pharoensis 5
pulcherrima
squamosa 8
Centrifugalization of blood
in trypanosomes 335
Cephal myia X
Cephen myia X
Cerat po III
Chi gerfl a X
Chir n mida III
Chir n mus 57
larva of 74
nymph of 11
Chlor form as fixative XIV
Chr m
m 34
Chrysops IV
Classification of Anopheline
lini 14
Cleaning of pipett
Clyp d hairs of larva 31
Clypeus of mosquito 143
Collection of mosquitoes 1
Col ur of Anopheline
lin 16
Cululi 55
Corethra 183
Corethra larva of 74 85
nymph of 11
Corethrina 174
Corrosive sublimate as
fixative XII
Counting by microscope
field 71
leucocyte 7
platelets 3
red cell 69
Crenations 15 28
Crescent male and female 23
Cross section of M 180
Culex 1 8
fatigans 16 1
larva of 74 76
nymph 88
post scutellum of 1 8
Culicid attitude 6
larva 8
palpi of 6
scales of 1
eggs of 11
Culicini 1 1
Curupi III
Cyst of
Cyst of malignant tertian
parasit 18
Cyclopod in genu
185 187
k d harm 1
m hip n tru
species 1

D

Diti *splendens* 34
Dan marks XIV
Dr h n globulin meter 73
Di dfym solution XII
Di se es 18

Dendromyia 18
Dermacentor 338
  reticulatus 330
Dermatobia x
D stoidea 177
Dilution formula for xvii
Diplococcus Pneumoniae and malaria 306
Diptera 57
  Cambridge Nat History 66
Dissection of salivary glands 113
  mosquitoes 103
Distribution of *Anophelina* 207
Diverticula of oesophagus 130
Dixa larva of 74 84
Domestic *Anophelina* 13
Dourine trypanosome of 357
Drepanidium ranarum 3 3
Dry season breeding places in 218
Dutton s membrane and *Filaria* 166 378

E

Eau de Javelle xii
Eggs *Anophelina* 69 seq
  classification by 190
  Culex 67
  examination of 68
  hibernation of 21
  Mansonia 7-
  Psorophora 7-
  Stegomyia 67 71
  Taeniorhynchus 68 7-
Ehrlich Biondi stain xvi
Embedding apparatus 40
Endemic area is of a country 267
  index 5
  index determination of 26
Endemic malaria to investigate 58
Endemicity malarial 5
Eosin B A 10
  and methylene blue xiv
Eosinophil leucocyte 13
Ephemera larva of 74
Epipharynx of mosquito 166
Eschatocephalus 339
Europe *Anophelina* of 08
Europeans how infected with malaria 65
European malaria to investigate 64

F

Farrant s solution xiii
Fat body of mosquito 133
Fat cells 110
Fecundation of *Anophelina* 18
Feeding experiments on birds 10
Female and male mosquitoes proportion between 219
Female crescent 9
Ficalbia 181
Filaria 364
  and Dutton s membrane 378
  characters of genus 368
  embryo development of 377
  embryo escape of 166
    in mosquito 1 3
  examination of blood for 37
  in birds 374
  in mosquitoes 375
  nocturna host of 05
  bancrofti 364 370
  demarquaii 368
  diurna 365

Filaria equina 373
evansi 373
gigas 3f8
haemorrhagica 373
immitis 373 376
irritans 373
lacrymalis 373
loa 3f5 371
megalhaesi 3f8 372
osleri 373
o .. ndi 3f7 371
palpebralis 373
perstans 3f 3
perstans and sleeping sickness 360
sec ndita 360 373
Filariae adults of 360
Filariasis and fleas 373
Finlaya 18
Fish trypanosomes in 34f
parasites in 325
Fixative chlor form as XIV
Fixatives ,
for blood XIV
for section XIII
Fixing solution X
Flagella 53
Flagellata of mosquitoes 1 2
Flagellate body of train 30
Flagellation of *halteridium* 318
Fleas XI
anatomy of XII
and *Filariasis* 373
and trypanosomes 348
Flea life history of XII
Flemming's solution XII
Fluke bl od u king wingles XI
Flight of *Anophelina* 215
Float f ea 21
Food of *Anophelina* 14
Formalin fixati XII
Formula for diluting XXII
Frill of ova 2 1
Frog trypanosomes in 345

G

Gal ziekte 360
Gamete female 35
mal 35
malignant tertian 3f
f halte idium 318
retr gression of 5f
simpl tertian 35
y ung f rm 3f
Garrapata tick 342
G stroph lus X
Genitalia of mosquitoes 1/1
Gilesia 178
Gl erin jelly XIII
Gl ssina 353
larva of 354
species of 354
ng f 34
Goeldia 183
G lgi s c le f 53
G wers haem gl bin meter 73
G dhamia 1/
G egarine of mosquitoes 1 3
G ernum t s f r bl d 285

H

Halrus IX
Haemagogus 182
Haemamoeba or *Danilewskya* 3 1
danilewskyi 31
gametes of 318
genus 314
Kochi 31
melanopherus 3
metchnikovi 321
murinu 321
relicta 314
ziemanni 321

Haemaphysalis 338
leachi 330 338
Haematapota IX
35
Haematein stain 50 XIV
Haematobia 351 X
Haematoidin in urine 289
Haematoporphyrin in urine 283
Haematoxylin eosin XV
Haemoglobinuria due to quinine 303
Haemoglobin to estimate 273
Haemogregarina bigemina 328
genus 32
lacazei 327
lacertarum 326
laverani 328
mesnili 327
ranarum 323
riedyi 325
splendens 324
stepanowi 325
Halteridium 317
flagellation of 318
gametes of 318
in sparrows 10
Hatching of larvae 4
Head of larva 229
parts of mosquito 163
Heat as fixative 9
Heller's test for blood 85
Heptaphlebomyia 180
Heptaphlebomyina 174
Hibernation of *Anophelina* 211
of eggs 21
of larvae 211
Hind gut of mosquito 131
Hippobosca VI
and trypanosomes VI 360
rufipes 360
Hippoboscidae 59
Histology of mosquito 139 155
Horse flies and Surra 349
Howardia 180
Human trypanosome 358
Hyalomma 340
Hypoderma V
Hypopharynx of mosquito 167
Hystrichopsylla IX

I

Identification of *Anophelina* 184 seq
Culicidae 17 seq
larvae 4
Index endemic 5
syphonic 51
India *Anophelina of* 09
quartan parasite in 68
Indian *Anophelina* larvae of 44 49
Infection of Europeans with malaria 65
Intermediate leucocyte 19
Iron reaction in malaria XVI
Isotonic point of blood 281
Ixodes 339
Ixodidae 335
Ixodinae 336

J

Janthinosoma 176
Javelle eau de XII
Joblotina 174 180
Joblotia post scutellum of 168

K

Karyolysus lacertarum 36
Kidneys in blackwater fever 31

L

Labellae of mosquito 166
Labium of 165
Lankesterella ranarum 33

Larvae abdomen of 33
antennae of 3 2
cannibal 63
classification of 1 40
clypeal hairs of 31
enemies of 63
examination of 23
food of
hatching of 4
head of 2 9
hibernation of 211
identification of 24
mental plates of 8
mounting of 50
of *Anopheles* 74 77 seq
of *Anophelina* 8
of *Chironomus* 74
of *Corethra* 4 85
of *Culex* 4
of *Culicidae*
of *Dixa* 74 84
of *Ephemera* 74
of Indian *Anophelines* 44
of *Mochlonyx* 84
of *Eucorethra*
of *Stegomyia*
of *Taeniorhynchus*
palmate hairs of 34
respiratory siphon of
Larvicide 83
Laveran p
Leaflet of palmate hairs 35
Legs hand l
Legs of mosquitoes
Leishman's stain
Length of life of *Anopheles* 57
Leucocytes counting of 70
differential count 41
dropsical 22
Leucocytes eosinophil
„ in blackwater fever 305
in malaria 274
in pneumonia
intermediate
in typhoid 79
large mononuclear 18 40
mast
normal values of 4
percentage of in malaria 75
pigmented 33
polymorphonuclear 18
small mononuclear (lymphocyte)
transitional
Leucocytic variation in malaria 4
Leucopenia in malaria 75
Leucotus 183
Lepidoptera VI
Lizard parasites 34
Löffler methylen blue
Ludia
Lutzia
Lymphocytes 18
Lynchia 51

M

Macrogamete 37 53
Madeira
Malaria ... 283
... 34
chromatin changes in 38
... 5

Malaria European to investigate 64
iron reaction in XVI
isotonic point of blood in 283
leucocytosis in 274 75
leucopenia in 75
parasite life history 57
parasite periodicity of 9-
percentage of leucocytes in 275
pigment in post mortem 305
post mortem changes 305
prevalence of 60
subsidiary signs of 39
the urine in 83
Malarial endemicity -5
map of 253
survey
map of 259
tissues to examine 43
to embed 44 48
to stain 50
Malaysia Anophelina of 09
Male Anopheles the 218
crescent 9
and female mosquitoes proportion between 219
Malignant tertian stippling 13
Malignant tertian parasite cycle of 98
Malpighian tubes of mosquito 13-
Mandibles of mosquito 167
Mansonia 179
eggs of 7
Mast leucocyte 19
Maxillary palps of mosquitoes 168
Media for mounting XIII
Megaloblast o
Megarhinus 175
Megarhinina 174
Melanin 27
Melanoconion 17-
Melophagus XI
Membrane of Dutton 166
Mental plate of larvae 3
Methaemoglobin in blackwater fever 85
tests for 87
Methylene blue XV
Microcytes 21
Microscope use of 14
Mid gut of mosquito 10f 108
Mikrogametocyte 37 33
Mochlonyx 183
larva 84
Mononuclear leucocytes
large 16 40
small 18
Monogony 56
Monkeys parasites in 31-
Mosquito abdomen of 171
Mosquito and yellow fever 24
antennae of 164
apparatus for collecting XIX
breeding out 95 96 157
capture of 95
collection of 156
clypeus of 163
dissection of 103
epipharynx of 166
fat body of 139
filaria embryo in 1-3
filaria in 375
flagellata of 1--
genitalia of 171

Mosquitoes, gregariousness of, 123
- head, parts of, 163
- hind gut of, 131
- hist[illegible] of, 135
- hypopharynx of, 167
- labella of, 166
- labium of, 165
- legs of, 170
- male and female, illustration [illegible]
- malpighian tubes of, 13[illegible]
- mandibles of, 16[illegible]
- maxillary palps, 16[illegible]
- maxillae of, 16[illegible]
- mid gut of, [illegible]
- muscular system of, 133
- nematodes of, [illegible]
- nervous system of, 13[illegible]
- oesophagus of, [illegible]
- [illegible] of, [illegible]
- post scutellum of, 16[illegible]
- pharynx of, [illegible]
- proboscis of, 16[illegible]
- pr[illegible] of, [illegible]
- pumping organ of, [illegible]
- reproductive system, 13[illegible]
- [illegible], 16[illegible]
- [illegible], 1[illegible]
- scutellum, 168
- scutum, 168
- [illegible], 143
- [illegible], 33
- [illegible], 122
- stomach, [illegible]
- [illegible]
- [illegible], [illegible]

Mosquitoes to feed on blood, 101
- take [illegible], [illegible]
- to kill, [illegible]
- to m[illegible], 15[illegible]
- trematodes in, [illegible]
- tracheal system, 134
- vascular system, 13[illegible]
- viscera, [illegible]

M[illegible] fly, IX

M[illegible] method of [illegible] mosquitoes, XIII

Muller, Bird, XI

Mus[illegible], IX

Mu[illegible], [illegible]

Muscular [illegible], 13[illegible]

M[illegible], [illegible]

M[illegible], XI

M[illegible], [illegible]

M[illegible], [illegible]

M[illegible], [illegible]
- [illegible]
- [illegible]
- [illegible]
- [illegible]
- [illegible], 53
- [illegible]
- *elegans*, [illegible]
- [illegible]
- [illegible]
- ungues, [illegible]
- *[illegible]*, [illegible]
- *[illegible]*, [illegible]
- *[illegible]*, [illegible]
- *lep[illegible]*, [illegible]

Myzomyia leucophyrus 196
listoni 193
longipalpis 195
ludlowii 195
lutzii 196
punctulatus 196
rhodesiensis 197
rossii 195
absence of sporozoits in 255
ungues of
tesselatum 196
turkhudi 197
ova of 6
Myzorhynchus genus 185 200
albotaeniatus 01
barcrofti 201
barbirostris 00
coustani 01
mauritianus 201
minutus 201
nigerrimus 01
paludis 201
plumiger 201
pseudo barbirostris 201
pseudopictus 01
mensis 201
umbrosus 01
umbrosus 01
zanus 01

N

Nematodes in mosquitoes 1??
Nemocera III
Nervous system of mosquitoes 137
Ngana trypanosome of 348
Nycteribiidae VI
Normoblasts 0
Nucleated red cells 0
Nympha of *Anophelina* 88
Nympha of *Chironomus* 91
of *Corethra* 91
of *Culex* 88
of *Taeniorhynchus* 83
syphon tubes 90
Nymphal stage 38
Nyssorhynchus genus 1 6 01
species 0
annulipes 05
deceptor 05
fuliginosus 20
jamesii 04
karwari 0
maculatus 03
maculipalpis v. *Indiensis* 204
masteri 05
metaboles 0
pretoriensis 04
stephensi 0
theobaldi 203
willmori 05

O

Ochromyia VI
Oedema in trypanosomiasis 36
Oesophagus diverticula of 130
of mosquito 1 ?
Oestrus V
Oil as larvicide 83
Oocyst 55
Ookinet 54
Ornithomyia VI
Ornithodorus 341
Orth's fluid XI
Osmic acid XIV
Ova floats of 1
frill of 1
of *Anophelina* 0
of *M. turkhudi* 6
to mount 7

Ova, types of 22
Ovaries of mosquito 1[illegible]0
Ovum 53
Ox warbles [illegible]

P

Pa[illegible]d time in *Anophelina* of [illegible]
Palmate hair leaflet of 35
of larvae 34
Palp banding of 190
Pangonia 353 IV
Pan plates [illegible]
Paraffin sections to fix VIII
wax in [illegible] 47
Parasites action of quinine on 3[illegible]
characters of 1[illegible]
crescent form [illegible]
crescent free [illegible]
cycle determination of [illegible]
in bats 3[illegible]
in birds 334
in blackwater fever 3[illegible]
in fish 3[illegible]
in lizard 3[illegible]
in monkeys 33[illegible]
in tortoises 3[illegible]
ring form [illegible]
malaria detection of 4
effect of quinine [illegible]
malignant tertian 3
malignant tertian sporulating 33
quartan 3 33
[illegible] 32 33
ring form 25
signet form 5
[illegible] 5
Parasite tertian (malignant) 3
(simple) 3
Pericardial cell 110
Periodicity of malaria parasite 32
Pharynx of mosquito 1[illegible]9
Phlebotomus [illegible] III
Phloroglucin XII
Phoniomyia 1[illegible]
Picric acid XII
Pigment in blackwater fever 305
of skin 1[illegible]
P. M. in malaria 3[illegible]5
Pigmented leucocytes 3[illegible]
Pipettes to clean [illegible]
Plasmodium genus 3[illegible]
bovis 3[illegible]
canis 33
equi 33
hominis 332
kochi 331
ovis 331
Plates 16[illegible]0
to count 13
Pneumonia leucocytes in [illegible]
Poikilocyte 33
Polar bodies [illegible]
Polychrome staining 6
Polychromophilus melanipherus 3[illegible]0
murinus 321
Polymorphonuclear leucocyte 1[illegible]
Post mortem changes in blackwater fever 311
Post mortem changes in malaria 3[illegible]5
Post scutellum of *Culex* 1[illegible]8
of *[illegible]* 165
of mosquitoes 1[illegible]
of *[illegible]* 1[illegible]

Prevalence of malaria 260
Proboscis of mosquito 165
Proteosoma 314
black spores 317
cycles of 315
in sparrows 107
vermiculi of 316
Proventriculus of mosquito 130
Psorophora 176
eggs 72
larvae 67
Psychodidae III
Pulex VIII
goniocephalus VIII
irritans VIII
and *Filaria* 373
serraticeps VIII
and *Filaria* 373
Pumping organ of mosquito 1 9
Pupation 238
Pyretophorus genus 185 198
atratipes 199
chaudoyei 199
cinereus 195
costalis 198
v. melas 198
costalis sporozoits in 56
jeyporensis 199
marshallii 198
merus 199
minimus 199
palestinensis 199
superpictus 198

Q

Quartan parasite 32 33
in Africa 266
in India 268
Quinine absorption of 299
action on parasites 30
and blackwater fever 303
Quinine effect on malaria parasite 4
elimination of 99
haemoglobinuria 303
in urine 291
Quotidian parasite 3 33

R

Rat trypanosome in 348
Razors sharpening of 46
Red cells counting of 69
large swollen 2
nucleated 20
penetration by sporozoits 118
with long processes
Reproductive system of mosquito 138
Respiratory syphons of larvae 75
Romanowsky stain 10 seq
Ross cycle of 5
Rhipicephalus 336
Runchomyia 18
Rhyncopsylla X
Rhyphidae 55
Ring form of parasite 25 7

S

Sabethes 18
Sabethoides 183
Safranin XI
Sahli s methylene blue XI
Salivary acini 13
glands dissection of 113
of *Anophelina* 119
of *Culicidae* 119
sections of 127
sporozoits in 117
structure 116
pump 167

Sand flies 58 III
Sarcophaga V
Sarcophila IX
Scales of *Anopheles* 186
of wing 1,4
varieties of 173
Schizogony 56
Schuffner dots 13 3
Screw worm larva V
Scutellum of mosquito 118
Scutum 118
Seasonal prevalence of
Anophelina 10
Sections of mosquitoes 123 seq
of salivary gland 1 7
Simulidae III 56
Sluse 181
Syphonic index 81
Syphon of larvae 81
tube of nymphae
Sleeping sickness and *F*
per tans 3
trypanosome 3 8
Slides to clean 2 23
Sole trypanosom m 357
Spirochaete *hilteridium* 102
protesoma 1
Species of *Uricchia*
Anopheles 1 1
Anophelina differ
entiation of 158
Arrisal 98
Cellia 98
Culex 1
Cs s 354
Myzomyia 1 13
Myzorhynchus 1
Nyssorhynchus 3
Separate tables
term ne 32
Pyretophorus 5 seq
Stethomyia 1 27
Spectroscopic test f blood 285
Spermath ca of mosquito 119
Spider flies VI
Spirillar fever and tick 343
in chicken 343
Spleen rate of 263
Sporoblast 55
Sporogony 56
Sporozoites in mosquitoes 1 2
Sporozoit 55
absence of in
M rossii 255
false appearance
of 108
in *M culicifacies* 2 5
in *M funestus* 5
in *P costalis* 5
in salivary gland 11
mutation 118
penetration of
red cell by 11
rate 3
1 time 115
Sporulating form of para
sites 31
Spotted fever 332
Stain VV
Stain Leishman 9 12
Romanowsky 12 seq
solubility VV
Staining Ins t role XXI
basophil 1
flagellate bodies 30
polychrom
sporozoits 118
Stegomyia 1
eggs 67 1
fasciata yellow
fever parasite in 34
genus 4
larva 85
Stenopteryx VI
Stethomyia genus 185 1

Stethomyia fasciata 177
  nimbai 197
Stomach of mosquitoes 104
  zygotes in 108
Stomoxys 351 X
Sublimate corrosive as fixative XII
Sugar in urine 290
Surra and horse flies 349
  trypanosome 349

T

Tabanidae 59 IV
Tabanus IX
Taeniorhynchus 179
  eggs 68 72
  larva 76 86
  nympha of 89
Tertian malignant gametes 36
  (malignant) parasite 3 33
  (simple) parasite 32
Texas fever parasite 3 8
Theobaldia 177
Ticks 33 seq
  anatomy of 334
  and spirillar fever 343
  classification of 335
Tick fever 341
Tipulidae 58
Toison's fluid 69
Tortoises parasites in 3 1
Toxorhynchites 176
Tracheal cells 109
  system of mosquitoes 134 seq
Transitional leucocyte 19
Trematodes in mosquitoes 1??
Trichocera 58
Trichoprosopina 174
Trypanoplasma 345
  borreli 363
Trypanoplasma danilewski 363
Trypanosoma 345
  and fleas 348
Trypanosome and *Hippobosca rufipes* 360
  and sleeping sickness 358
  and tsetse fly 348
Trypanosomes blood examination in 36
Trypanosoma brucei 348
Tropanosoma brucei and *trypanosoma evansi* compared 356
Trypanosoma carassii 346
  cobitis 346
  eberthi 346
  evansi 349
  equinum 356
  equiperdium 357
  gambiense 358
  lewisii 348
  rotatorium 345
  soleae 347
  theileri 360
  transvaaliense 360
  ugandense 358
Trypanosomes dimension of 361
  human 358
Trypanosomes in frogs 345
  rats 348
  inoculation of 36
  multiplication of 361
Trypanosome of *Dourine* 357
  mal de caderas 356
  Surra 349
Trypanosomiasis oedema fluid in 36
Trypanosomiasis centrifugalization of blood 358
Tsetse fly 349

Tsetse fly trypanosome 318
Types of ova 2
Typhlopsylla IX
Typhoid leucocytes in 2,,
Widal reaction in 2,,

U

Ungues of M. funesta 170
specific value of 170
M. rossii 170
Uranotaenia 181
Urine bile pigment in 280
bilirubin in 283
haematoidin in 28,
haematoporphyrin in 28,
methaemoglobin in 28
in blackwater fever 31(
in malaria 83
quinine in 231
sugar in 80
urobilin in 8,
Urobilin test for 87
Urobilinuria in black water fever 311

V

Vacuoles 15
Vascular system of mosquitoes 136
Vein (cross) of M. rossii 18,
Verde, Cape XI
Vermicule X
Vermicule of Haemoproteus 31(
Vermiculus 5(
Vermipsylla X
Verrallina 191
Viscera of mosquitoes 103

W

Weight and measures XXI
Widal reaction in typhoid 29
Wild *Anophelina* 13
Wings fringe of 158
Wing of *Anophelina* 169
scales of 174
Wings spots on 158
Wingless flies 3(
Wyeomyia 18
post-scutellum of 168

Y

Yellow fever 341
parasite development of 342

Z

Zenker fluid XI
Zygote 53
Zygotes malignant tertian 111
proteosoma 111
quartan 111
simple tertian 111
in thick (,
to mount 112

PRINTED BY DONALD FRASER, 3 MOUNT STREET, LIVERPOOL

PUBLICATIONS OF THE
LIVERPOOL SCHOOL OF TROPICAL MEDICINE

Published for
The University Press of Liverpool
by
LONGMANS, GREEN & CO
39 Paternoster Row, London
New York and Bombay
1903

MEMOIR IV

Report of the Malaria Expedition to Nigeria (1900) By th[illegible] [illegible] (by D[illegible]) [illegible]

MEMOIR V PART 1

First Progress Report of the Campaign against Mosquitoes in Sierra Leone (1901) By M[illegible] R R[illegible] FRCS DPH FRS [illegible]

MEMOIR V PART 2

Second Progress Report of the Campaign against Mosquitoes in Sierra Leone By M[illegible] [illegible]

MEMOIR VII

Report of the Yellow Fever Expedition to Para (1900) By H[illegible] D[illegible] M[illegible] FRCS [illegible] W[illegible] M[illegible] MB [illegible] this expedition) [illegible] 6d

MEMOIR VIII

Report on the Sanitary Conditions of Cape Coast Town With [illegible] By M[illegible] MB ChB [illegible]

MEMOIR IX

Report on Malaria at Ismailia and Suez By R[illegible] R[illegible] FRCS DPH FRS CB [illegible]

MEMOIR X

Report of the Malaria Expedition to the Gambia By J E D[illegible] MB ChB [illegible] 6d

MISCELLANEOUS

Notes on Sanitary Conditions obtaining in Para by th[illegible] Mer[illegible] [illegible] Exp[illegible] Pr[illegible]